AMINO ACID MALNUTRITION

RUTGERS UNIVERSITY
Bureau of Biological Research

THE ANNUAL CONFERENCES ON
PROTEIN METABOLISM

1952 *Protein Metabolism, Hormones and Growth*
80 pages

1953 *Some Conjugated Proteins*
73 pages

1954 *Serological Approaches to Studies of Protein Structure and Metabolism*
97 pages

1955 *Some Physiological Aspects and Consequences of Parasitism*
90 pages

1956 *Some Aspects of Amino Acid Supplementation*
85 pages

Amino Acid Malnutrition

Edited by William H. Cole

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FOREWORD

As a regular feature of its program of studies on protein metabolism, the Bureau of Biological Research at Rutgers, The State University, has sponsored annual conferences on some aspect of that general theme since 1945 At each conference a small number of investigators, from four to eight, have been invited to present the results of their recent studies against the background of other contributions to the subject *Informality of presentation has been encouraged, and time has been al-lowed* for free discussion of each report The conferences have been open to all interested persons who registered for them The average attendance has exceeded two hundred

The first three conferences dealt with dietary proteins and protein derivatives, particularly therapeutic protein hydrolysates The next four conferences considered caloric intake, certain amino acids, peptides enzymes, vitamins, and minerals in relation to health and disease The proceedings of the first seven conferences were distributed only to those who attended

The next five conferences were concerned with protein metabolism, hormones and growth conjugated proteins, serological approaches to protein structure and metabolism physiological aspects and consequences of parasitism, and dietary amino acid supplementation The proceedings of those conferences have been published to make the material available to an audience larger than the one in attendance This policy is continued for the 1957 conference and will probably be followed for future conferences

During recent years biologists and clinicians have been intensively studying the nutritive requirements of plants animals, and man Thousands of studies have been reported on plants, bacteria fungi protozoa, a few invertebrate animals, and many mammals Perhaps the largest number has dealt with bacteria, the rat, the dog, and man Emphasis has shifted from caloric intake to vitamins to the quality and relative amounts of protein carbohydrate and fat required, to balances between them, and finally to detailed considerations of balances among the proteins and their constituent amino acids Attention has also been given to the interacting roles of hormones, vitamins, and minerals

In several studies on animals where the experimental conditions are more easily controlled than on humans, evidence has been secured that damage to the structure and function of the body from unknown causes may often be corrected by a specific modification or supplementation of the diet Thus specific dietary constituents may act as therapeutic

agents in preventing and curing diseases which were formerly not considered related to dietary intake Manifestations of malnutrition may therefore be more widespread than are now recognized

With the increasing attention given to human protein nutrition during recent years, some progress has been made in demonstrating that certain human disturbances and diseases may be cured by proper dietary supplements For example, kwashiorkor is now recognized as one type of protein-deficiency disease, easily and quickly cured by adding milk to the diet Detailed studies of this disease in Jamaican and Guatemalan children are presented in this volume In other areas of the world where food is scarce, or where particular food habits interfere with adequate nutrition several individuals and groups are making careful studies to determine the proper protein or amino acid supplementation required For certain countries this may mean the formulation of an entirely new diet composed of materials available but not now used Some of the work is being advised and supported in part by the United Nations through the Food and Agriculture Organization, the World Health Organization, the Children's Fund (UNICEF) and the Institute of Nutrition of Central America and Panama (INCAP) Private foundations are also providing financial aid to such studies, which promise the solution of one of the most difficult problems facing the world — i e , inadequate nutrition One of the first steps in this direction has been the authoritative survey of food production in eight countries of the world contrasted to that in the United States presented herewith

Another frontier now possible of exploration by dietary tools is mental health The rapid progress in the discovery and use of the so-called "wonder drugs of the nervous system" has not been accompanied by equal advances in the understanding of the central nervous system's metabolism As an introduction to such knowledge an original and critical summary of the amino acid and protein metabolism of the brain is included in this volume Some outstanding differences from the metabolism of other tissues and organs are disclosed Although no interpretation is yet possible, it is safe to assume that correlations between specific metabolic processes in the brain and aspects of behavior will be possible

In addition to the subjects mentioned above the Thirteenth Annual Conference on Protein Metabolism devoted to Amino Acid Malnutrition, included an account of the repletion of protein-depleted animals as a background for human tests and a discussion of mixtures of pure amino acids as dietary supplements

Acknowledgement is thankfully made to the authors of the papers for their assistance in preparing this book for prompt publication

WILLIAM H COLE
Chairman
Conference Committee

January 30, 1957

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REPLETION OF DEPLETED PROTEIN RESERVES IN ANIMALS[1]

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Protein malnutrition is associated with a loss in protein reserves in the blood and soft tissues of the body *(1-4)* These reserves are the tissue proteins which rise and fall with corresponding increases or decreases in the dietary protein intake, and they are believed to reach an optimum in the presence of an adequate quantity and a proper balance of dietary amino acids Depletion in protein reserves has numerous striking effects upon the metabolism and welfare of an animal Since many protein reserves are enzyme systems, protein malnutrition alters rates of reactions in intermediary metabolism If a vitamin is part of the coenzyme, the alterations may also be characteristic of a vitamin deficiency Data have been presented, for example, which demonstrate the reduced ability of the depleted animal to detoxify or oxidize organic radicals, a reduction which is also associated with riboflavin and pantothenate deficiencies *(5)* In general, labile protein reserves are associated with numerous metabolic functions, being important, for example, to maintenance of water balance *(6)* to formation of antibodies *(3 7)* to healing of wounds *(8)* to mechanisms for oxidation and detoxication *(5)* and to correction of dietary deficiencies associated with periods of restricted food intake *(4)*

There is still some question, however, concerning the magnitude of development of the tissue protein reserves for optimum welfare of the animal and the effect of dietary proteins of various nutritive values upon this development The following experiments were designed to answer these questions in part Dogs were depleted in reserves by feeding a protein free diet and the rate of repletion studied in animals fed casein or wheat gluten The casein was also supplemented with methionine and wheat gluten with lysine to determine the effect of increasing the nutritive value of these proteins The results of repletion in depleted animals were compared with some previous studies on the effect of the same dietary proteins upon growth in young dogs

METHODS

Adult dogs were fed a protein-free diet which contained the vitamin, mineral, and caloric requirements estimated for this animal under

[1] These researches were supported in part by grants in aid from the National Cancer Institute and the New Jersey Heart Association

TABLE II

AVERAGE DATA OBTAINED FROM A STUDY OF 16 DOGS FED A PROTEIN FREE DIET FOR 4 WEEKS

	Urinary		
Weeks Depletion	Urea Nitrogen	Amonia Nitrogen mg/day/kg body weight	Creatinine
1	162	22 5	20 4
2	111	22 5	21 0
3	94	22 1	21 4
4	93	21 5	19 3

constant value in all depleted dogs Possibly the magnitude of reduction in excretion of urea to this constant value is one estimate of the quantity of reserves available for utilization under conditions of stress The constancy of excretion of urea after initial depletion of reserves and of creatinine may be interpreted to represent essential catabolic activities that are of endogenous origin *(15 16)*

In the following studies on repletion, the response of the animals was estimated primarily in terms of plasma proteins and nitrogen balance two variables which are used extensively to measure loss and gain in protein reserves Under the experimental conditions, the serum albumin concentration decreased with loss in body nitrogen in a semilogarithmic manner as illustrated in Figure 1 Body nitrogen loss was calculated by assuming the over-all protein content of the dog to be approximately 16 per cent Plasma globulin concentration, on the other hand, at first increased slightly in concentration with depletion in reserves, but then decreased to a subnormal concentration at a point of maximum reduction in body nitrogen At this point of maximum loss, the animal is approaching a critical stage where repletion is difficult or even impossible The initial rise in globulin concentration is the result of a fall in plasma volume, the total circulating globulins either remaining unchanged or actually decreasing with depletion in reserves This tendency for certain globulins to be reduced in depleted animals is illustrated by the data in Table III. The fact that a globulin fraction does not decrease on depletion, however, does not mean that such a fraction is not labile and cannot be reduced The total circulating γ globulins, for example, may be reduced upon depletion in protein reserves but under the stress of infection, which often passes through a colony, this fraction may increase This variability in response of γ globulin is an example of the capacity of the body to draw upon reserves to synthesize specific proteins when metabolism shifts in that direction

TABLE I

DIET FED TO DOGS

Ingredient	Grams A	Grams B	Vitamins	mg/2400 gm agar diet
Protein	0	250	Thiamin	2 0
Sucrose	229	0	Riboflavin	7 6
Dextrose	387	366	Nicotinic acid	16 0
Dextrin	187	187	Calcium pantothenate	13 0
Lard	153	153	Pyridoxine	1 0
Salt mixture	17	17	Choline	1000
Agar	27	27	2 methylnaphthoquinone	0 0006
Water	1400	1400	Alpha tocopherol	30 0
			Biotin	0 6
			Folic acid	0 6
			Vitamin A	55 000 units
			Vitamin D	11 000 units

"normal" conditions *(9)* This is illustrated by A in Table I The diet was prepared as follows about two-thirds of the water was added to a kettle, together with the agar the mixture being heated until the agar dissolved The lard was cut into chunks, added to the hot agar solution, and the heat removed While the lard melted, the carbohydrate, protein (if it was to be added), and salts were mixed and then added to the agar solution Water was supplied to make up the amount recorded in Table I The liquid was stirred until it thickened, the vitamins included, and the diet poured into pans to gel and to be stored in the refrigerator The dogs were fed the protein-free diet equivalent to 80 calories/day/kg of body weight for a period of four weeks Then for 28 days the animals were fed a diet containing protein such as the one illustrated by B in Table I. Urine and feces were collected so that nitrogen balances could be calculated for the whole experiment Urea and ammonia were determined by the Conway method as modified by Steinetz *(10)* Blood volumes and extracellular fluid determinations *(11 12)* electrophoretic analyses for plasma protein *(13)* and plasma aldolase determinations *(14)* were made periodically

DEPLETION OF PROTEIN RESERVES

Depletion of protein reserves is usually accompanied by a reduction in catabolic activity as reflected by a fall in the excretion of urea nitrogen (see Table II) The greater the labile protein reserves the higher the initial excretion of urea, but the excretion drops to a low and fairly

NUTRITIVE VALUES OF THE CASEIN AND WHEAT GLUTEN

Casein and wheat gluten were chosen as two dietary proteins to study the effects of nitrogen intake upon repletion of protein reserves because they represent a high and a low nutritive value, respectively, and yet both are deficient primarily in one, though different, essential amino acid The nitrogen balance index of casein, for example, in the normal dog with full protein reserves was determined to be 0 74 *(17)* Casein is deficient in sulfur amino acids for the dog so that adding methionine to casein raised the index to 1 0, equivalent to the value for egg proteins The index for casein in the depleted dog was 0 84, a much higher value than in the normal animal, such augmentation in the utilization of dietary amino acids being common to the depleted animal The index for wheat gluten was 0 44 in the normal dog but increased to 0 70 when the reserves were depleted Wheat gluten is deficient primarily in lysine so that the addition of this amino acid in concentrations about equal to the amount found in casein raised the index in the normal dog to 0 73 *(17)*

NITROGEN BALANCE DURING REPLETION

Feeding 0 2 gm casein nitrogen/day/kg of body weight to the depleted dogs for 28 days produced a small but constant positive balance of approximately 0 05 gm nitrogen/day/kg These data, illustrated by the solid circles in Figure 2, may be interpreted to mean that this amount of casein nitrogen is just a bit over that needed to maintain the animal in the depleted state with very little filling of the protein reserves The circles with vertical bars record data obtained while feeding 0 6 gm of casein nitrogen/day/kg body weight in a diet equivalent to 80 calories/day/kg The slope of the line drawn through these points may be interpreted to represent the rate of filling of the protein reserves the area under the curve is an estimate of the grams of nitrogen retained during repletion The line drawn through the open circles in Figure 2 measures the increased rate and amount of filling of the reserves in dogs fed a higher intake of 1 12 gm casein nitrogen/day/kg and approximately 140 calories/day/kg The circles with cross bars demonstrate the improvement in nutritive value obtained while feeding 1 12 gm casein nitrogen/day/kg supplemented with an optimum amount of methionine *(18)* In general, these data demonstrate the increased rates of filling of the protein reserves and amounts of nitrogen retained, both values being correlated with a rise in nitrogen intake and with supplementation to correct for a deficiency in an essential amino acid

The results of feeding approximately 0 6 gm of wheat gluten nitrogen/day/kg and 80 calories/day/kg to depleted dogs for 28 days are recorded by the circles with vertical bars in Figure 3 The open circles illustrate the increased rate and amount of nitrogen retention obtained while feeding 1 1 gm of wheat gluten nitrogen/day/kg at the higher caloric intake The circles with cross bars record the still

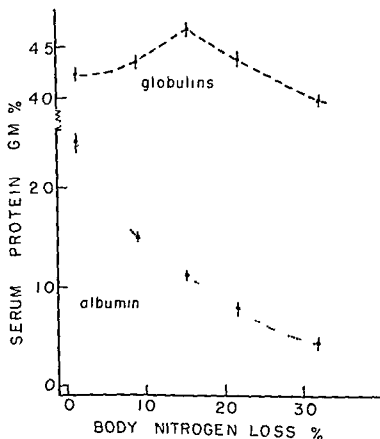


Figure 1 Grams per cent of serum albumin and serum globulin correlated with loss in body nitrogen in dogs fed a protein free diet

TABLE III

AVERAGE DATA OBTAINED FROM A STUDY OF 16 DOGS FED THE PROTEIN FREE DIET OVER A PERIOD OF 28 DAYS

Weeks Depletion	Plasma Albumin	Plasma globuline					
		α_1	α_2	α gm/kg body weight	β_1	β_2	γ
0	1 32	0 35	0 33	0 45	0 57	0 46	0 27
2	0 75	0 43	0 24	0 33	0 41	0 42	0 35
4	0 54	0 39	0 27	0 29	0 34	0 37	0 37

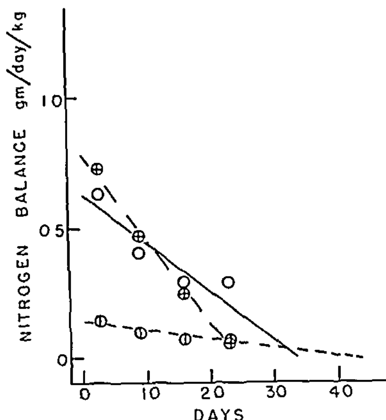


Figure 3 Nitrogen balance in gm/day/kg body weight correlated with days of repletion in dogs fed wheat gluten nitrogen 0 6 gm nitrogen/day/kg illustrated by circles with vertical bars 1 1 gm wheat gluten nitrogen/day/kg by open circles and 1 1 gm nitrogen/day/kg supplemented with 1 3% lysine by circles with cross bars

for wheat gluten This equilibrium condition was reached after feeding the casein nitrogen for 24 days, but 44 days was estimated for the attainment of equilibrium while feeding the wheat gluten Feeding approximately 1 gm of either casein or wheat gluten nitrogen/day/kg and 150 calories/day/kg body weight added 9 5 gm nitrogen/kg to the protein reserves, thereby establishing a new condition of equilibrium with greater reserves The rate of filling at the higher intake was again slowest in dogs fed the unsupplemented wheat gluten The data in Table IV illustrate also the increased rate of filling of protein reserves associated with supplementation of casein with methionine and of wheat gluten with lysine

These data demonstrate that it is possible to develop approximate steady states, represented by nitrogen equilibrium, in depleted animals and in animals in various stages of repletion Possibly some protein reserves may be repleted more rapidly than others so that each partial stage of repletion represents a new physiological state This possibility

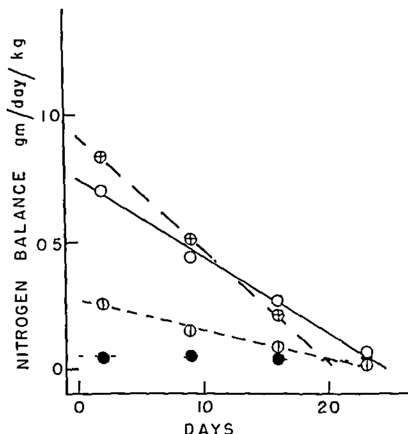


Figure 2 Nitrogen balance in gm/day/kg body weight correlated with days of repletion in dogs fed casein nitrogen 0 2 gm nitrogen/day/kg body weight illustrated by solid circles 0 6 gm nitrogen/day/kg by circles with vertical bars 1 12 gm nitrogen/day/kg by open circles and 1 12 gm casein nitrogen/day/kg supplemented with 0 3% methionine by circles with cross bars

greater increase associated with the feeding of 1 3 per cent lysine These results demonstrate that unsupplemented wheat gluten, with a lower nutritive value than casein, fills the protein reserves at a much slower rate than the milk protein Supplementing with lysine, however, increases the rate to be equivalent to casein

REPLETION OF PROTEIN RESERVES

These differences in rates and amounts of filling of the reserves, illustrated in Figures 2 and 3 can be expressed quantitatively, as in Table IV The rates are the slopes of the lines and the amounts of nitrogen retained are the areas under the lines Either protein source fed at approximately 0 6 gm nitrogen/day/kg body weight and 80 calories/day/kg developed an approximate equilibrium condition with a relatively low nitrogen retention of 3 2 gm/kg for casein and a 2 9 gm/kg

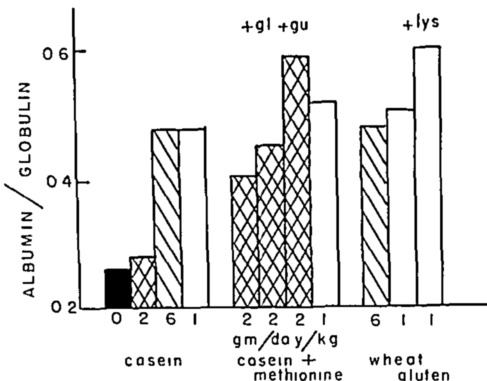


Figure 4 Albumin/globulin ratios correlated with nitrogen intake in gm/day/kg body weight The black bar illustrates the ratio in the protein-depleted dogs before reple tion Results from feeding 0 2 gm casein nitrogen then supplemented with methionine (M) or with methionine + glycine (Gl) or with methionine + guanidoacetic acid (Gu) are represented by cross hatched bars feeding 0 6 gm nitrogen by bars with slanted lines feeding 1 gm nitrogen by open bars

than feeding casein with methionine, results which emphasize the specific effect of methionine plus guanidoacetic acid upon the ratio Thus this combined supplement increased the albumin/globulin ratio to values equal to or higher than normal even though the dogs were still in the depleted state, results which emphasize the specific effect that a dietary protein source can have upon regeneration of one type of tissue protein *(20)*

The data in Figure 4 demonstrate also the relatively greater repletion of the albumin/globulin ratio in dogs fed wheat gluten than in those fed unsupplemented casein These ratios were determined after 28 days, when dogs fed wheat gluten had not reached equilibrium, and repletion in nitrogen reserves was far below those fed casein The albumin/globulin ratios, however, in dogs repleted with wheat gluten, were equal to or higher than the ratios observed in animals fed the milk protein Possibly the relatively higher concentration of sulfur amino acids in wheat gluten than in casein *(1)* is correlated with the greater effect of the wheat protein upon the ratio Feeding wheat gluten supplemented with lysine produced the maximum ratio of 0 6, a result

TABLE IV

AVERAGE VALUES OBTAINED WHILE FEEDING DIFFERENT DIETARY PROTEINS TO GROUPS OF PROTEIN DEPLETED DOGS (4 6 DOGS TO A GROUP) FOR A PERIOD OF 28 DAYS

Dietary Protein	Nitrogen Intake	Initial Nitrogen Balance	NB x 10³ / days	Calculated Nitrogen Retention
	gm/day/kg B W			gm/kg B W
casein	0 6	0 26	11	3 2
wheat gluten	0 55	0 14	3	2 9
casein	1 12	0 71	30	9 5
+ methionine	1 12	0 84	45	9 5
Wheat gluten	1 06	0 63	19	10 0
+ lysine	1 10	0 72	34	9 7

was revealed in part by the study of repletion of plasma proteins The black bar in Figure 4 illustrates the albumin/globulin ratio in the protein-depleted dogs just before repletion The first series of bars in Figure 4 demonstrate that the albumin/globulin ratio did not increase after feeding 0 2 gm of casein nitrogen/day/kg for 28 days but, as would be expected, feeding either the higher intakes of 0 6 or 1 12 gm of casein nitrogen during this period increased the ratio Unexpectedly, however, the maximum increase of the ratio during repletion with casein was reached while feeding 0 6 gm instead of the higher intake of 1 12 gm of nitrogen Supplementing the higher casein nitrogen intake with methionine (see last open bar over casein plus methionine) raised the ratio still further to 0 52 a result which suggests a special function of methionine in repletion of plasma proteins

Adding an optimum amount of methionine to the low casein diet increased the nitrogen balance but slightly more than resulted from feeding the unsupplemented casein, but this slight increase in nitrogen retention was accompanied by a marked rise in the albumin/globulin ratio from approximately 0 26 in the depleted state to 0 41 (see Figure 4) Previous experiments in our laboratories suggested that the addition of guanidoacetic acid (Gu) with the methionine to the low casein diet would specifically increase the ratio still further *(19)* Protein-depleted dogs, therefore, were fed 0 2 gm casein nitrogen/day/kg supplemented with DL-methionine and guanidoacetic acid (each being added on the basis of 0 7 per cent of the dry diet) Even though the nitrogen balances were not increased significantly above values obtained during feeding casein supplemented with methionine alone, the albumin/globulin ratio was increased markedly to 0 59 while feeding the diet to which both methionine and guanidoacetic acid had been added Feeding casein supplemented with methionine plus glycine (Gl) increased the ratio but little more

Adding methionine and guanidoacetic acid to the low casein diet resulted in a rise in plasma volume to values equal to or greater than normal even though the animals were still far from being repleted in protein reserves There was also a rapid correction of extracellular fluid toward normal

Plasma aldolase activity, similar to plasma albumin concentration, has been found to increase or decrease with the rise or fall in protein reserves of the body The decrease in activity of this enzyme upon depletion is illustrated in Figure 6, where the first white bar over C records the aldolase activity in normal dogs and the black bars represent

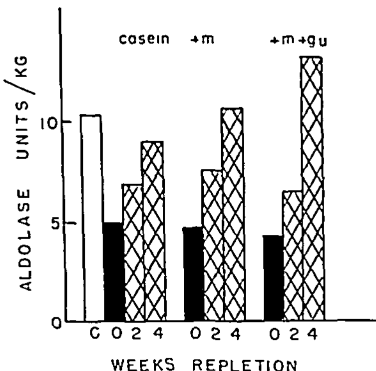


Figure 6 Plasma aldolase activity in normal dogs (C) after depletion (O) and during repletion in animals fed 0.2 gm casein nitrogen/day/kg body weight then supplemented with methionine (M) or with methionine + guanidoacetic acid (Gu)

the activities in the depleted state Increased aldolase activity resulting during the period of feeding 0 2 gm casein nitrogen/day/kg body weight is illustrated by the cross-hatched bars Thus, even though this low nitrogen intake had no effect upon repletion of plasma albumin plasma aldolase activity was raised toward values found in repleted animals Again a marked effect of supplementing the casein with methionine, or still better with methionine plus guanidoacetic acid, was demonstrated by the augmentation of aldolase activity

which can be correlated with the over-all high nutritive value of the supplemented wheat protein

Feeding even small amounts of nitrogen such as 0 2 gm/day/kg to a depleted dog may have more value than can be indicated by the low positive nitrogen balance produced Certainly the animals improved in appearance and activity during these four weeks of feeding the restricted casein intake The data plotted in Figure 5 demonstrate that the extra cellular fluid was returned toward normal in animals fed the low nitro gen diet even though the plasma volume remained low as in the depleted

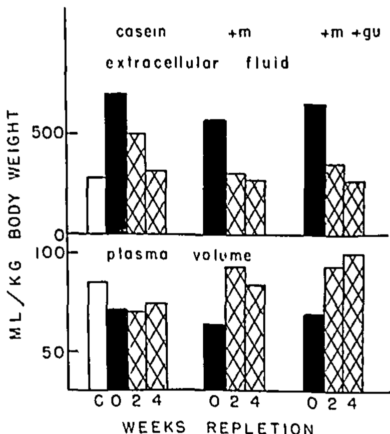


Figure 5 Extracellular fluid and plasma volume correlated with weeks of repletion in dogs fed 0 2 gm casein nitrogen/day/kg body weight then supplemented with methionine (M) or with methionine + guanidoacetic acid (Gu) The bars with slanted lines record normal values the black bars illustrate data from depleted dogs

state This shift in extracellular fluid is thought to be associated with repletion of certain proteins which increase the water holding capacity of the tissues The data in Figure 5, however, illustrate the marked rise in plasma volume that is associated with the increase in albumin/globulin ratios recorded for the supplemented casein diets in Figure 4

Adding methionine and guanidoacetic acid to the low casein diet resulted in a rise in plasma volume to values equal to or greater than normal even though the animals were still far from being repleted in protein reserves There was also a rapid correction of extracellular fluid toward normal

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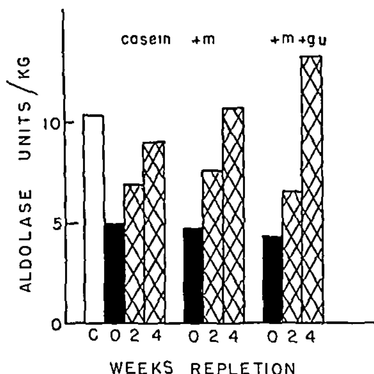


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GROWTH

These data on repletion of depleted protein reserves in adult dogs demonstrate that the rates and paths of repletion vary with the nitrogen intake and the nutritive value and type of dietary protein source Similar data have been obtained while studying nitrogen retention in growing puppies The same diet was fed to the puppies for growth as was used for repletion of depleted adults (diet B in Table I) The dietary protein sources were varied to study the effects of egg proteins, casein, and wheat gluten on growth and nitrogen retention The nitrogen retained during growth was almost three times greater in the puppies fed egg protein than in those fed wheat gluten Puppies fed casein retained somewhat less nitrogen than those fed egg protein but retained more than twice as much as the animals fed wheat gluten *(20)* This is shown in Table V All animals, however, grew at about the same rate in

TABLE V

AVERAGE INCREASE IN BODY WEIGHT (B W) PER GRAM OF NITROGEN INTAKE (I) AND AVERAGE POSITIVE NITROGEN BALANCE (B) PER GRAM OF NITROGEN INTAKE (I) IN BEAGLE PUPPIES DURING FAST GROWING PERIODS (Data from reference *21*)

Protein Source	$\frac{B\ W}{I}$	$\frac{B}{I}$
Whole egg	9 0	0 50
Casein	9 8	0 43
Wheat gluten	8 8	0 18

weight, the grams gain in weight per gram of nitrogen intake being approximately 9 in puppies whether they were fed egg proteins casein, or wheat gluten, but physiologically and in body composition they were different The animals fed wheat gluten were low in body protein but high in fat stores compared to those fed the other two protein sources The puppies fed egg proteins were lean and active those fed wheat gluten were obese and less active These and other observations on growth or repletion of tissues emphasize the importance of establishing an optimum relationship between caloric and protein intake to keep a proper balance between fat stores and lean body mass

It is appropriate to end this discussion of dietary protein requirements for repletion and growth of tissue proteins by emphasizing the importance of balance between all the constituents of the diet Water *(21)* inorganic elements *(22)* amino acids *(23)* vitamins *(24 25)* and fat and carbohydrates *(26 27)* in sufficient quantity and proportions are essential to the development and well being of each tissue The body is well

adapted to correct for many dietary errors but not all of them and it is the important function of the nutritionist to discover and correct those errors which lead to inefficiency or premature death of the living system

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S^{35} METHIONINE UPTAKE IN PROTEIN-DEPLETED JAMAICAN CHILDREN

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In Jamaica, as in most tropical countries, protein malnutrition is common, and varies greatly in its severity Usually only in young children does it represent a danger to life the recently weaned child has a very low protein intake at a time of life when its protein requirements are relatively very great The Jamaican diet is deficient both in quantity and quality of protein, usually the majority of it is of vegetable origin *(1)* The clinical syndromes which arise in these malnourished children are called *kuashiorkor* and *marasmus* and intermediate types are very commonly seen *(2)* As in the case in other countries, the Jamaican versions of these syndromes show local characteristics but there is no doubt that the fundamental etiology is the same as that of the clinically similar conditions found in protein-malnourished populations throughout the world Dr Holt has suggested that the local variations are due to local characteristics of the amino acid pattern *(3)*, and if this is so, the condition may fairly be called *amino acid malnutrition* We have no evidence of specific amino acid deficiency or excess in our cases, however, so it is more accurate to term the condition *protein depletion* The belief that the children are protein depleted is supported by the dietary histories, by the response to refeeding protein and by direct analysis of liver and muscle biopsy samples *(4)*

This condition of protein malnutrition is the most serious and widespread nutritional disorder known to medical and nutritional science *(5)* Mortality is about 10 to 30 per cent *(6)* even when the best regimes of treatment are used, and the reason for this mortality is now known In view of the seriousness of this problem and the tremendous weight of research that has been brought to bear upon it in recent years it is all the more interesting, and humiliating that the best available guide to enable us to predict whether a severe case will live or die is whether or not it can be persuaded to smile Of the available biochemical tests which can be used to follow or predict the course of recovery the two which we have found most useful are the plasma albumen concentration and the plasma pseudocholinesterase activity When the prognosis as suggested by these tests, however differs from that based on clinical impression it is usually the biochemistry which is the more misleading The need for a reliable and objective measure of the severity of a case and of its rate of progress is great since without such a measure it is

impossible to compare the efficacy of different therapies, or of the same therapy in different series of cases

Radioactive amino acids have been used with great effect to investigate other disorders of protein metabolism, so we thought, perhaps naively, that they might be used to provide some insight into the enigma of the protein-depleted child The choice of methionine as the tracer was based on expediency *rather than biochemistry It was impracticable* in our Unit to use isotopes requiring a mass spectrometer for analysis, and the use of C^{14} C-labeled amino acids is not permitted in young children in view of the long radioactive half-life S^{35} has a convenient half-life of 87 1 days, and also has the advantage that it leaves the body almost entirely by the urine Methionine is an essential amino acid and there is some evidence that it is low in the diets which produce kwashiorkor It is given in tracer quantities in an attempt to obtain an undistorted picture of the metabolism of the natural free methionine circulating at the time of injection, and so by analogy of the behavior of all circulating free amino acids

METHODS

Two of the children reported on in this paper were admitted to the ward of the Medical Research Council Tropical Metabolism Research Unit and one to the pediatric ward of the University College Hospital under Dr Eric Back The children in this Unit were fed on admission on skim milk at a dilution which would be tolerated without vomiting When full-strength skim milk was well tolerated Mixture A was given, and in convalescence Mixture E The composition of these feeds is given in Table I

TABLE I

COMPOSITION OF DIETS
(gm or ml per 100 gm mixture)

Diet	Casilan (gm)	Dried Skim Milk (gm)	Sugar (gm)	Oil (ml)	Water (ml)	Protein (gm)	Calories
Full strength skim milk		10	10	-	80	3 4	80
Mixture A	5	5	2	3	85	5 7	70
Mixture E		12	2	5	81	4 1	93

Each child was injected twice, the first time when it had ceased to be acutely ill or edematous but was not showing clinical signs of rapid recovery, and the second time after the phase of rapid recovery had been entered

The injection solution contained 0 5 mg S^{35} DL-methionine and 4 0

mg Evans blue (T1824) per ml and 1 ml per kilogram was given intra venously Before injection 2 ml of venous blood was withdrawn, and this was used for the determination of total protein, albumen, and globulin concentrations, and in the case of a second injection to measure the residual activity from the previous injection The dose to be injected was taken from a weighed bottle into a separate syringe, and after injection the syringe was rinsed out in 25 ml of 0 9 per cent saline, and the concentration of dye in the washings was measured The dose injected was calculated from the weight loss of the bottle less the amount recovered in the washings One ml samples of capillary blood were taken from a heel prick at 1/4, 1 2, 4 6 and 8 hours after injection The serum was separated from these samples by centrifugation the yield was approximately 0 5 ml

From the 1/4 and 1 hour samples 0 04 ml was taken and diluted with 0 20 ml of 0 9 per cent saline The concentration of Evans blue in these samples was used to calculate a volume of distribution of the dye, termed *plasma volume*

From each of the samples 0 2 ml was used to determine the activity in protein-free plasma This was done by precipitation of the protein with 0 04 ml of 30 per cent trichloracetic acid (TCA), and measurement of the activity in an aliquot of the supernatant after centrifugation The precipitate was then washed twice with 5 per cent TCA and redissolved in 0 15 ml of normal NaOH This gave an approximately 5 per cent solution of total serum protein 0 04 ml aliquots of this solution were prepared for counting, and similar aliquots were used for the measurement of nitrogen content by Kjeldahl digestion

Finally 0 2 ml of the samples were used for the separation of the albumen and globulin fractions by a modification of the cold-methanol method of Pillemer and Hutchinson *(7)* The protein fractions were precipitated and redissolved and counted in the same way as total protein

The design of the counter used and the technique of preparing samples for counting has been described *(8)*

Total 24-hour collections of urine were made on alternate days from one of the cases, and the nitrogen content was determined by Kjeldahl digestion An aliquot of urine was mixed with an equal volume of a 10 per cent solution of gum ghatti and the activity of the mixture was measured in the same way as for protein solutions

RESULTS

The course of a typical case of marasmus is summarized in Figure 1 On admission this child was eleven months old he had no teeth and was unable to sit up His birth weight had been 5 1/2 pounds and he had been weaned at four months onto a diet of oat porridge, saltine biscuits and one tin of condensed milk a week No parasites or pathogenic organisms were found in the stool and the Mantous test was negative There was no evidence of infection or electrolyte upset so the case may

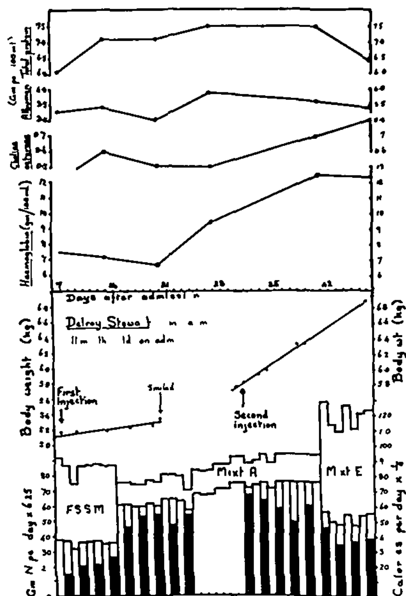


Figure 1 Clinical determinations on a child with marasmus (DS in Table II) Daily nitrogen output in the urine x 6 25 and the calories ingested per day x 0 1 are shown by the columns at the bottom of the figure body weight is shown by the black dots through which an average line is drawn hemoglobin choline esterase albumen and total protein in the plasma are shown by the curves in the upper half of the figure

be considered to be one of pure protein undernutrition The first injection was made on the seventh day after admission when, although food intake was good the child was not gaining weight nor making convincing progress clinically On the twentieth day the child smiled for the first

mg Evans blue (T1824) per ml and 1 ml per kilogram was given intravenously Before injection 2 ml of venous blood was withdrawn, and this was used for the determination of total protein, albumen, and globulin concentrations, and in the case of a second injection to measure the residual activity from the previous injection The dose to be injected was taken from a weighed bottle into a separate syringe, and after injection the syringe was rinsed out in 25 ml of 0 9 per cent saline, and the concentration of dye in the washings was measured The dose injected was calculated from the weight loss of the bottle less the amount recovered in the washings One ml samples of capillary blood were taken from a heel prick at 1/4, 1, 2, 4, 6, and 8 hours after injection The serum was separated from these samples by centrifugation the yield was approximately 0 5 ml

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were on a protein-free diet and were injected with four times the dose of radioactivity that was used in the children It can be seen that the more severely depleted dogs show a higher activity in plasma protein than the normal dog The depleted dogs were also found to excrete urine containing more labeled sulfur per mg nitrogen than normal dogs This effect is illustrated in Figure 3

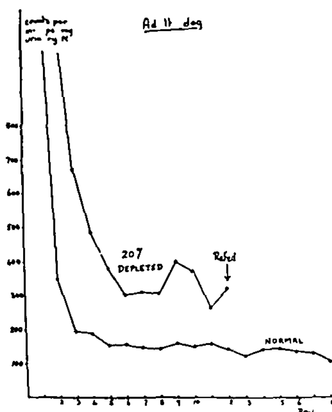


Figure 3 The excretion of labeled sulfur measured by counts per minute per milligram of urinary nitrogen by a normal dog (lower curve) and one which had been experimentally depleted of 20 per cent of his body protein (upper curve) The clinical condition of the depleted dog on the twelfth day after injection made it necessary to feed normal protein

The uptake of methionine by the marasmic child was in accordance with the results obtained in the dogs, and is illustrated in Figure 4 The upper curve shows the activity incorporated into plasma protein after the second injection The same dose of radioactivity was given on each occasion It can be seen that the plasma protein reaches a much higher peak activity when the child is still severely depleted than it does when the child is recovering rapidly and has to some extent made good his protein lack The excretion of labeled sulfur in the urine also follows

time and thereafter made rapid progress clinically The second injection was made on the thirty-first day after admission, when the child was obviously recovering rapidly and showing satisfactory weight gain Food intake throughout the period shown in this figure was satisfactory the protein intake was between 50 and 75 gm per day after the fourteenth day, and the caloric intake between 700 and 1,300 gm per day The urinary nitrogen is shown by the solid black columns at the foot of the figure Since stool nitrogen was not measured, it is not possible to give figures for nitrogen absorption or balance

The curves showing the incorporation of labeled methionine into plasma protein have been interpreted in the light of preliminary studies done on experimentally protein-depleted dogs The important results of these experiments may be summarized in two diagrams Figure 2 shows some incorporation curves in normal and depleted dogs which

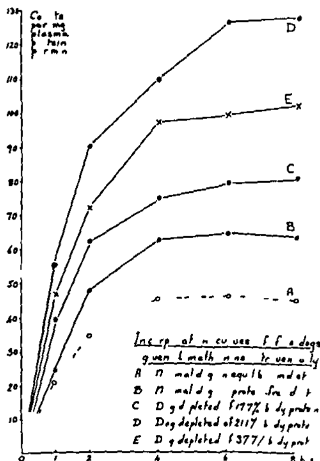


Figure 2 The incorporation curves of five dogs given S^{35} L methionine intravenously (4×10^6 counts per minute per kilogram of body weight) Normal dogs A and B were on an equilibrium diet dogs C D and E had been depleted to the extent of 17 7 21 1 and 37 7 per cent of their body protein respectively

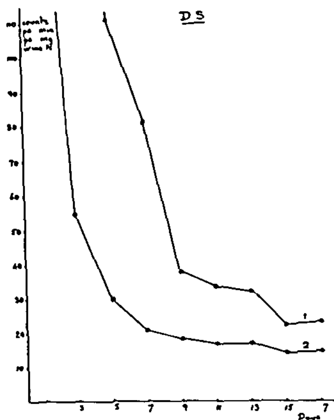


Figure 5 The excretion of labeled sulfur in the urine of the marasmic child referred to in Figure 1 The upper curve (1) shows the excretion after the first injection and the lower curve (2) after the second injection when the child was recovering (Compare Figure 4)

severely ill and was brought for medical attention partly because she had scabies over her entire body and partly because she was not thriving Food intake was good from the day of admission, and the failure to gain weight during the first two weeks was no doubt partly due to the gain in tissue mass being masked by the loss of excess fluid Clinical edema had disappeared by the fifth day The low serum protein concentrations on admission were partly due to a large plasma volume The first injection was made on the ninth day and the second on the thirty-ninth day after admission

The uptake of radioactivity into plasma protein in the second case is shown in Figure 7 Again the peak after the first injection is higher than after the second but the difference does not appear to be as striking as it was in the case of the marasmic child

The incorporation curves for another marasmic child are shown in Figure 8 This child had a birth weight of 9 pounds reached 14 pounds at seven months and then started to lose weight and was admitted at the

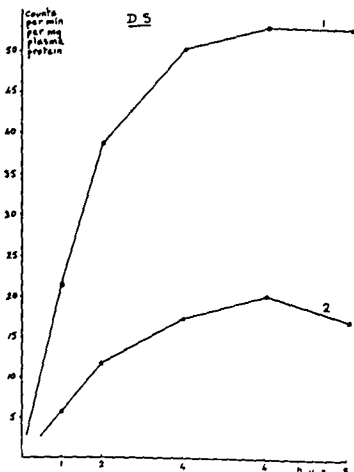


Figure 4 Incorporation of S^{35} DL methionine into plasma protein after intravenous administration to the marasmic child referred to in Figure 1 The upper curve (1) shows the uptake after the first injection on the seventh day after admission and the lower curve (2) after the second injection on the thirty first day when the child was recovering rapidly The amount injected in each case was 1×10^6 counts per minute per kilogram of body weight

the pattern which was found in the dog experiments This is illustrated in Figure 5

The diet weight gain, and some biochemical findings in a typical case of kwashiorkor are illustrated in Figure 6 Urine collections in this case were incomplete and are not recorded On admission the child had edema of the feet and a liver enlarged to 4 cm below the costal margin in the mid-line Analysis of a needle biopsy showed that fat accounted for 23 per cent of the wet weight of the liver The child had been taking her feeds well, but her diet had been composed of corn-meal porridge, banana, bush teas, biscuit, and one tin of condensed milk per week shared between the child and her mother The child was not

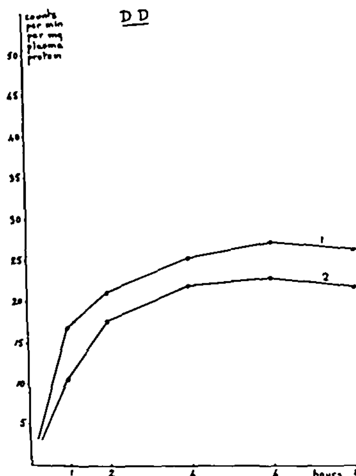


Figure 7 Incorporation of S^{35} DL methionine into plasma protein after intravenous ad ministration to the child with kwashiorkor referred to in Figure 6 (DD in Table II) The upper curve (1) shows the uptake after the first injection on the ninth day after admission and the lower curve (2) after the second injection on the thirty ninth day The amount injected in each case was 1 x 10 counts per minute per kilogram of body weight

found for his wasting apart from underfeeding He took full-strength skim milk well, but it was two and a half weeks before his weight had increased by one pound The first injection was made at this time, and the second a month later when the child was clinically greatly improved and showing a satisfactory weight gain Again the curve after the earlier injection shows a higher peak than that after the later one

The interpretation of these results requires caution The activity attained in a protein pool after injection of a radioactive tracer depends on three factors the number of radioactive molecules which enter the pool the number of molecules in the pool to start with, and the rate at which labeled molecules in the pool are exchanged for unlabeled ones from elsewhere

We have some information concerning the rate at which plasma albu-

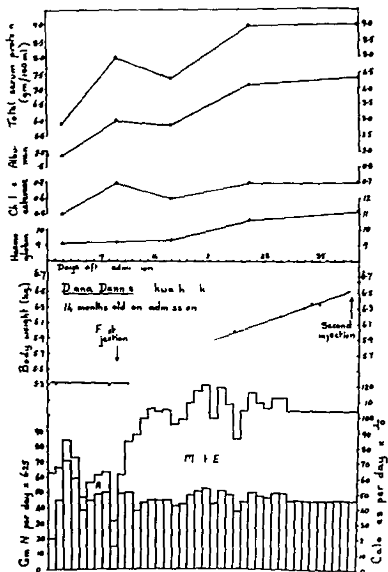


Figure 6 Clinical determinations on a child with kwashiorkor (DD in Table II) Daily nitrogen output x 6 25 and calories ingested per day x 0 1 are shown by the columns at the bottom weight gain is shown in the middle hemoglobin choline esterase albumen and total serum protein are shown respectively in the upper half of the figure The initial plasma volume was relatively high giving low serum protein values There was edema of the legs on admission which had disappeared by the fifth day

age of eleven months weighing only 8 pounds The mother insisted that the child had been given half a pint of goat s milk daily, but it is difficult to accept this as true On admission the child had an abscess in the groin which was incised and healed well but no other obvious cause was

This calculation for the three cases discussed is shown in Table II It can be seen that the case of kwashiorkor, D D , had a smaller plasma volume per kilogram at the time of the second injection than at the first, since the body weight had increased more rapidly than the plasma

TABLE II

PROPORTION OF INJECTED ACTIVITY INCORPORATED INTO INTRAVASCULAR PLASMA PROTEIN OF THREE CHILDREN FOLLOWING THE FIRST AND SECOND INJECTIONS OF S^{35} METHIONINE

	D D		D S		R M	
Injection	1	2	1	2	1	2
Peak plasma protein activity (cpm/mg)	26 9	22 7	5[illegible] 7	21 6	31 0	23 6
Body weight (kg)	5 30	6 65	5 20	5 61	4 38	5 32
Plasma volume (ml)	219	250	137	229	168	20[illegible]
Plasma volume (ml/kg)	40 3	37 6	26 4	40 8	38 3	38 0
Plasma protein (gm/100 ml)	8 0	9 0	6 1	7 5	6 1	7 0
Plasma protein (gm/kg)	3 23	3 39	1 61	3 06	2 34	2 66
% dose in plasma protein	8 7	7 7	8 5	6 6	8 2	6 3

volume The protein concentration had also increased so that each kilogram of body weight contained 3 39 gm plasma protein at the time of the second injection and 3 23 gm at the time of the first For each kilogram body weight 1 x 10^6 cpm were injected so this is labeling a bigger pool the second time than the first Even so it requires 8 7 per cent of the injected dose to give the observed activity of 26 9 cpm/mg protein after the first injection and only 7 7 per cent to give 22 7 cpm/mg after the second.

The first case of marasmus (D S) shows a large drop in activity on the second injection, but this is largely accounted for by the increase in the size of the plasma protein pool The second case (R M) has a smaller drop in activity but a smaller change in pool size Again both cases show that a larger proportion of the dose entered the plasma protein pool on the first occasion than on the second When a similar calculation is done for the normal and experimentally protein-depleted dogs a similar difference is found

In a depleted child who is being refed protein, a given protein pool may receive new protein either from dietary sources or from another pool of body protein Both sources may affect the level of radioactivity in the pool and it is impossible to distinguish between changes in radioactivity due to one source or the other In the dog on a protein-free diet the problem is simpler, since all protein is necessarily endogenous

The depleted dog on a protein-free diet excretes urine of a higher activity per mg nitrogen than does a normal dog this urine is presumably derived from protein of higher activity It has been shown that the depleted dog has plasma protein of high activity but the plasma protein pool is not by itself large enough to support the urinary loss One must

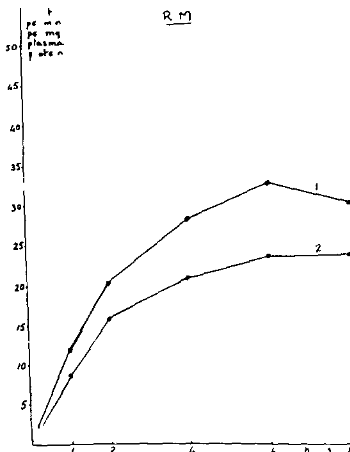


Figure 8 Incorporation of S^{35} DL methionine into plasma protein after intravenous administration to a child with marasmus (RM in Table II) The upper curve (1) shows the uptake after the first injection on the sixteenth day after admission and the lower curve (2) after the second injection on the forty eighth day The dose on each injection was 1 x 10 counts per minute per kilogram of body weight

men exchanges with albumen in the extravascular space from a consideration of the behavior of albumen marked with radioactive iodine or Evans blue Such experiments have not shown any significant difference between the rate of loss from the intravascular pool whether the child is depleted or not In interpreting the curves for uptake of methionine, therefore the rate of loss of labeled protein into the lymph space has been assumed to be the same after both injections The size of the pool into which the labeled molecules are going can be calculated from the plasma volume which has been determined with Evans blue and from the serum protein concentration The third variable can therefore be calculated from a knowledge of the peak activity reached and a figure given for the proportion of the injected activity which has been found in intravascular plasma protein

The relative levels of radioactivity in the pools are represented by the number of horizontal lines per cm The pool sizes and relative levels of activity shown are based on dog experiments, but such data as we have on depleted children suggest that the type of redistribution shown applies to human cases also It appears that on depletion the active pool becomes smaller and more active, and the structural protein becomes even more inert than in the normal animal

Obviously this work is only a beginning but the method of study with the newer tracer techniques seems likely to be a fruitful one We are learning that the malnourished child who weighs 10 pounds at the age of one year is not metabolically a miniature version of the normal child of one year, nor is he the same as a normal child who weighs 10 pounds at the age of three months Some at least of the metabolic differences can be demonstrated by tracer methods, and we may hope that these methods will lead us to a better understanding of the nature and significance of the changes which occur in response to protein malnutrition

SUMMARY

There is no reliable test of the severity of protein depletion or indicator as to prognosis The absence of such a test makes therapeutic trials hazardous and difficult to interpret

In experimentally protein-depleted dogs and in malnourished children a change from the normal distribution of protein anabolism has been demonstrated by use of radioactive methionine as a tracer

It is suggested that such tracer studies may provide information not otherwise obtainable concerning the essential physiology of protein depletion and thereby assist in the development and assessment of new methods of treatment

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suppose, therefore, that other protein pools exist which, like plasma protein are metabolically more active in the depleted state and take a larger share of the injected activity Since the total activity injected per kilogram is constant the existence of some protein of higher than usual activity implies that some other protein is of lower than usual activity, the distribution of activity has changed

Any description based on tracer studies such as these of the protein metabolism of the intact animal is subject to severe limitations The available equations are far fewer than the unknowns, so if we are not to abandon the attempt as hopeless we must make some simplifying assumptions A simple model, therefore, which will serve to illustrate the redistribution of protein anabolism which occurs on protein depletion is shown in Figure 9 Body protein is considered in three pools

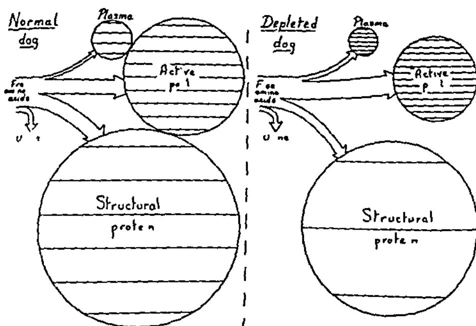


Figure 9 Diagrams for normal and protein depleted dogs showing the pathways of the ingested amino acids into the urine the plasma protein pool the active protein pool and the structural protein pool of the body and how the relative levels of radioactivity vary in the three pools (See text for details)

plasma protein, "active" protein, and "structural protein There is evidence from many animal experiments that nonplasma protein behaves roughly as a mixture of about 25 per cent of a rapidly metabolising component and 75 per cent of a relatively inert component *(9)* The left hand diagram represents a normal animal the right-hand diagram represents an animal which has lost 30 per cent of its body protein

in short supply in most areas where the syndrome is a problem Despite the great amount that can be done to improve animal production in many underdeveloped areas, there is no reasonable prospect that this will furnish sufficient quality protein soon enough or at a sufficiently low cost to provide a satisfactory solution

As has been recently pointed out *(6)* there are a number of contributing factors, such as intercurrent infection and particularly diarrheal disease, which serve to precipitate kwashiorkor in children who are already basically malnourished Environmental sanitation and other measures aimed at the control of these precipitating causes can do much to reduce the incidence of acute kwashiorkor but cannot in themselves solve the basic problem which is protein malnutrition

For these reasons research workers in a number of countries and the specialized agencies of the United Nations concerned with nutrition - the World Health Organization (WHO) the Food and Agricultural Organization (FAO) and the United Nations Children Emergency Fund (UNICEF) - have turned to the problem of making suitable combinations of vegetable protein available for infant feeding in underdeveloped areas in which protein malnutrition is a serious problem

While such a mixture is theoretically possible the task is not simple Much more information is needed about the amounts of amino acids required and the adverse effects of possible amino acid imbalances as well as further data on the biological availability of the amino acids in many of the most likely mixtures New vegetable-protein combinations should not be tested in human beings until after painstaking animal testing Frequently these facilities are not available in the area where the greatest need and interest in the development exists There must be assurance that the mixture contains no toxic factors inherent in the ingredients or resulting from the proposed method of processing In addition, it is axiomatic that practical mixtures for human feeding in underdeveloped areas must be inexpensive, made principally from locally available ingredients, palatable, capable of easy storage and transportation and acceptable as a food for infants and young children to the parents of those for whom it is intended

Succeeding sections discuss the initial experiences of the Institute of Nutrition of Central America and Panama (INCAP) in its efforts to develop such a mixture and present some preliminary observations on the use of the amino acid pattern of the provisional reference protein recently proposed by the Committee on Protein Requirements of FAO *(7)*

BASIS FOR THE DEVELOPMENT OF AN INCAP VEGETABLE MIXTURE

As the result of nine years of work in the Instituto Agropecuario Nacional of Guatemala (IAN) by one of the authors (R.L.S) in developing animal rations based on local plant resources *(8-12)* and seven years of study of the nutritive value of the foods of Central America

3

VEGETABLE PROTEIN MIXTURES FOR THE FEEDING OF INFANTS AND YOUNG CHILDREN[1]

Nevil S Scrimshaw, Institute of Nutrition of Central America and Panama (INCAP)[2], Robert L Squibb, Instituto Agropecuario Nacional (IAN)[3], Recardo Bressani, Moises Béhar, Fernando Viteri, and Guillermo Arroyave (INCAP)

Protein deficiency, or more specifically, the lack of the right amounts and proportions of the essential amino acids, is now widely understood to be the major nutritional problem, particularly among young children, in most of the world s underdeveloped areas The word *kuashiorkor* as used for a syndrome which includes retarded growth and maturation, edema, alterations in the skin and hair, apathy, ano rexia, diarrhea, and a variety of accompanying biochemical and physio logical changes, has become part of our professional vocabulary In Latin America it is known as *Sindrome Pluricarencial Infantil* Studies are in progress in many parts of the world, not only to understand the nature of kwashiorkor, but also to find practical means of preventing it in the countries in which it is now prevalent

So much has recently been written about the characteristics and treatment of kwashiorkor *(1-5)* that no attempt is made to review these aspects of the problem in the present paper The purpose is rather to discuss the suitability of different foods for the prevention of kwashi- orkor with special reference to products of vegetable origin This ap proach must contend with the problem of the poor quality of vegetable protein from simple sources and the degree to which it can be improved through suitable combinations or even through direct supplementation with synthetic amino acids

There is no doubt that sufficient milk or other protein of animal origin, given to young children whenever the supply of mother s milk becomes inadequate for their needs, would effectively prevent the oc- currence of kwashiorkor Unfortunately, animal protein is costly and

[1] Various parts of this work have been assisted by Grant No 981 from the National Institutes of Health, and by funds from the National Research Council E I Du Pont de Nemours and Company and by the World Health Organization of the United Nations

[2] INCAP is a cooperative institute for the study of human nutrition supported by the Governments of Costa Rica El Salvador Guatemala Honduras Nicaragua and Panama and administered by the Pan American Sanitary Bureau Regional Office of the World Health Organization

[3] IAN is the National Agricultural Institute of Guatemala

Vegetable Mixture 8, while undoubtedly capable of further improvement and of variation to meet the current availability and price of its ingredients, was considered sufficiently promising to be tested in rats and chicks The animal trials were so successful as to justify human-feeding experiments, and these have also proved highly satisfactory

The composition of INCAP Vegetable Mixture 8 is as follows

	%
Dried corn masa	50
Sesame meal	35
Cottonseed press cake	9
Torula yeast	3
Kikuyu-leaf meal	3

The source of each of these ingredients is as follows

Dried Corn Masa *(Zea mays)*

Corn masa is made by heating whole corn to boiling in a solution of 0 5 to 1 per cent calcium hydroxide and holding it at this temperature for 30 to 45 minutes After it has cooled, the water is poured off and the whole kernels ground in a mechanical mill *(18)* It is then dried to make a flour In the initial rat, chick, and human trials reported, corn masa made locally was dried for the purpose For the latter trials, including all the metabolic balance studies, tortilla flour was purchased in Mexico [4] The composition of this product is given in Tables I-III It should be pointed out that the amino acid composition *(19 20)* and the carotene content *(21)* will vary significantly with the variety of corn employed

TABLE I

PROXIMATE COMPOSITION OF INCAP VEGETABLE MIXTURE 8

	Moisture %	Protein %	Fat %	CHO %	Calories / 100 g	Crude Fiber %	Ash %
Corn Masa	9 6	8 1	3 2	77 7	372	1 2	1 4
Sesame Meal	7 0	41 0	33 0	5 5	483	5 2	13 5
Cottonseed Press Cake	5 4	54 0	4 8	29 1	376	2 6	6 7
Kikuyu Leaf Meal	10 5	18 0	4 0	57 8	339	19 0	9 7
Torula Yeast	7 0	50 0		35 5	342	0 5	7 5
Mixture 8	8 3	25 1	13 7	46 3	503	3 2	6 6

[4] Minsa produced by license from the Banco Nacional de Mexico

and Panama by INCAP *(13-15)*, considerable basic information was available as to potential ingredients of an all-vegetable mixture for human feeding Because of the very large part which corn already plays in the Central American diet, and because a practical commercial procedure existed for preparing lime-treated corn in the form of tortilla flour, it was decided to use this flour as the basis for the mixture and to look for a combination of other products of plant origin which would be locally available and which would improve sufficiently its nutritive value

Soybean, which would otherwise have been a major ingredient of the mixture is not at present grown in Central America in significant quantities The background of successful use of sesame meal in improving the protein quantity and quality of animal rations and its local availability indicated that this meal should be a key ingredient The large quantity of cottonseed meal available as a by-product and its known value as a protein concentrate for animal feeding, together with the availability of a process for preparing a safe and palatable cottonseed meal for human consumption led to the use of this product

Since the objective was to develop a food mixture which did not require supplementation with manufactured nutrients, a food source of vitamin-A activity was necessary Experience showed that a number of the leafy forage plants such as kikuyu grass *(Pennicetum clandestinum)* desmodium *(Desmodium intortum)* and ramie *(Boehmeria nivea)* contained high vitamin-A activity and were locally available in Central America *(16 17)*

No combination of these proposed ingredients would, however, supply sufficient riboflavin, niacin, or ascorbic acid Because the raw materials for the manufacture of yeast are available at low cost in Central America, the incorporation of a small amount of this ingredient was chosen as the means of introducing an adequate content of B-complex vitamins Since ascorbic-acid rich fruits and fruit juices are readily available, no attempt was made to include this vitamin in the mixture

Although this mixture was not intended to serve as the sole food of the child and in theory need not serve as anything more than a protein supplement, it was highly desirable to have it as complete nutritionally as practical Even with the acceptance of this protein rich mixture by people in underdeveloped areas as appropriate food for their young children, the other dietary components are likely to be primarily carbohydrate in nature In such cases neglect of the vitamin content of the mixture could result in clinical deficiencies

FORMULA FOR INCAP VEGETABLE MIXTURE 8

All available data were assembled about the various potential ingredients and on the basis of calculated nutritive value economic feasibility, and palatability, formulas were developed which seemed progressively more promising A combination identified only as INCAP

TABLE III

AMINO ACID CONTENT OF INGREDIENTS OF INCAP VEGETABLE MIXTURE 8

Amino Acid	Corn %	Sesame %	Cottonseed %	Torula %	Alfalfa[1] %	Calculated Total mg Amino Acid	mg Amino Acid/ g N	Provisional Protein mg Amino Acid/ g N	Score
Arginine	0 42	3 19	5 64	1 82	0 83	1910	478	-	
Histidine	0 43	0 59	1 32	1 00	0 25	490	122		
Isoleucine	0 40	1 89	1 88	1 83	0 61	1100	276	270	100
Leucine	0 86	3 15	2 94	2 68	1 03	1900	475	306	100
Lysine	0 28	1 00	2 15	2 62	1 06	780	195	270	72 (83)[2]
Methionine	0 16	1 14	0 70	0 67	0 04	560	245	270	91
Cystine	0 08	0 86	0 80	0 34		420			
Phenylalanine	0 40	2 90	2 60	1 51	0 68	1520	603	180	100
Tyrosine	0 36	1 54	1 31	1 64		890			
Threonine	0 28	1 15	1 76	1 82	0 54	770	193	180	100
Tryptophan	0 04	0 59	0 59	0 51	0 22	310	78	90	86
Valine	0 44	1 36	2 46	1 98	0 87	1010	253	360	70 (100)[2]

[1] Figures are for alfalfa since no data are yet available for kikuyu
[2] When score is based on analysis of complete mixture rather than calculation from ingredients

TABLE II

VITAMIN AND MINERAL CONTENT OF INGREDIENTS AND OF INCAP VEGETABLE MIXTURE 8[1]

Ingredient	Carotene mg/100 g	Thiamin mg/100 g	Riboflavin mg/100 g	Nicotinic Acid mg/100 g	Calcium mg/100 g	Phosphorus mg/100 g	Iron mg/100 g
Corn masa	0 15[2]	0 40	0 12	2 31	131	145	0 8
Sesame meal		1 02	0 47	10 60	2154	1324	107 0
Cottonseed press cake		0 14	0 36	4 24	149	1535	10 6
Kikuyu leaf meal	23 80[3]	0 35	1 89	4 62	231	188	21 0
Torula yeast		27 00	10 00	100 00	900	2000	18 0
Mixture 8	0 8	1 41	0 61	8 39	867	740	40 0

[1] Ascorbic acid content negligible
[2] \aries with type of corn used
[3] Based on average value of other leaf meals

content of these ingredients are listed in Table II Except for the torula yeast and the kikuyu grass, the values are based on INCAP analyses In the quantities ordinarily consumed by young children when the vegetable mixture is the sole source of protein, it contains satisfactory amounts of all known essential nutrients except ascorbic acid

The amino acid content of the mixture as compared to the amino acid pattern for a provisional reference protein is shown in Table III (7) It will be apparent that the mixture contains as much of all the essential amino acids as the reference protein per gram of nitrogen, except for lysine, valine, and possibly tryptophan Since the final factor determining protein value is the availability of the essential amino acids to the animal organism, and not their content as determined microbiologically on the hydrolyzed protein, the mixture was tested in rats before attempting to improve its theoretical value when compared with the reference protein Its protein score by calculation would appear to be approximately 70, if lysine is considered to be limiting, and 85 if valine is taken to be the limiting amino acid

All the ingredients except the yeast and the leaf meal are, of course, heat treated in the course of their preparation, but for feeding to children further cooking before serving is desirable to assure a completely sanitary product and to improve palatability Most of the mixture fed to children has been cooked with hot water in the same manner as oatmeal using a double boiler for convenience It was served as a hot gruel flavored with sugar, similar to the *atoles* which are commonly used in Central America The mixture was also made into a very palatable dessert by cooking it with oleomargarine, sugar, vanilla, and a small amount of water in a double boiler, it can be served either hot or cold Although only these two methods of preparation have been used thus far in the feeding trials with children, there are obviously a wide variety of recipes which can be developed for its use, and there is the possibility of preparing the mixture in a completely precooked form

ANIMAL TRIALS

Work with Rats

Rats fed the vegetable mixture as the sole source of protein with added vitamins and minerals showed very satisfactory growth which was not improved by the addition of lysine, under the conditions of the experiments The results in Table IV are representative of six trials employing 272 rats in 26 groups, the complete animal tests of the mixture will be reported elsewhere

It will be seen that the mixture as fed supplied approximately 25 per cent protein and that the growth was good There was an apparent improvement in feed efficiency, however, when lysine was added When the percentage of protein in the experimental diet was reduced by the addition of sucrose to 19 2 and 12 8 per cent respectively, final weights of 151 and 120 grams were observed in three weeks In the latter case,

Sesame Meal (*Sesamum orientale*)

Locally produced sesame oil meal was used in the initial animal trials, but in later experiments a flour specially prepared for human consumption was obtained from the American Sesame Products, Inc of Paris, Texas [a] In making this product the grain was dehulled, cleaned to maximum purity, steamed, roasted, and then crushed before being ground The composition of this product is given in Tables I III The temperature of the material entering the press did not exceed 230°F The sesame from which the meal was prepared is the variety known as Renner No 1, and was grown in western Texas, and the irrigated areas around Lubbock and Artisia, New Mexico

Cottonseed Meal (*Gossypium hirsutum*)

The initial animal trials were made with a cottonseed press cake prepared locally For all the later work, a cottonseed flour, made for human consumption, was obtained through UNICEF from the Traders Oil Mill Company of Fort Worth, Texas To prepare it, prime cotton seed was delinted and dehulled and the hull-free meats flaked in conventional equipment The fresh, flaked meats were not heated above 275°F prior to screw pressing The free gossypol content did not exceed 0 045 per cent Other analytical values are given in Tables I-III

Torula Yeast (*Torulopsis utilis*)

Torula yeast, designated as Type 100 manufactured by the Lake States Yeast Corporation of Rhinelander Wisconsin, was obtained through the courtesy of Charles Bowman and Company, New York In addition to 50 per cent protein, this product contained 0 27 mg of thiamin, 0 1 mg of riboflavin, and 1 0 mg of niacin per gram, according to the manufacturers specifications

Kikuyu-leaf Meal (*Pennicetum clandestinum*)

Fresh young kikuyu grass averaging 45 cm in height, grown in the grounds of the Instituto Agropecuario Nacional in Guatemala City, was collected and dried for approximately 48 hours in warm moving air at a temperature not exceeding 140°F The dry grass was then ground as finely as possible in a Wiley mill Its average β-carotene content was 23 8 mg per cent Analytical values for its content of other nutrients are given in Tables I-III

COMPOSITION OF THE MIXTURE

The approximate composition of the mixture and its individual components is given in Table I and the values for the vitamin and mineral

[a] Through the courtesy of Mr John Kraft and Mr Roy H Anderson

TABLE V

EXAMPLES OF GROWTH IN CHICKS FED INCAP VEGETABLE MIXTURE 8[1]

Trial		Feed Efficiency[4]	Initial Weight g	Final Weight g
1A	8	2 80	42	200
1B	Modified 8[3]	2 84	42	217
1C	8 + 0 2% lysine	2 19	42	298
1D	8 + 0 4% lysine	2 05	42	369
2A	8 + 0 5% lysine	2 40	42	405
2B	Modified 8[3] + 0 5% lysine	2 39	42	431

[1] 24 chicks per group 5 weeks with 25% protein
[2] Mixture was supplemented with minerals and vitamins to meet chick requirements
[3] Whole ground corn substituted for lime treated corn (masa)
[4] Grams fed per grams gained

good growth when the added lysine needed by the chick was provided Although the tests were qualitative in nature only, it was reassuring that excellent growth was obtained in rats even when the level of protein in the diet was reduced to 12 per cent Furthermore, the chick growth observed was comparable in some trials to that expected with the commercial rations The mixture proved palatable and no signs of toxicity were encountered Although long-term trials in rats extending over more than one generation and trials in baby pigs are contemplated before the mixture is recommended for commercial production, the data available appeared to justify carefully supervised human trials with hospitalized children

HUMAN TRIALS

The initial trials consisted of giving small quantities of the mixture to children receiving a mixed diet to determine its palatability, and to make sure that it would not produce diarrhea or other gastrointestinal disturbances The children accepted the mixture well and it was then given as the sole source of protein to two children who had completely recovered from kwashiorkor and later to three children who had recovered from the acute phase but were not yet ready for discharge from the hospital The case reports on these five children have recently been published (b) and can be summarized by saying that the results were highly satisfactory even in a child given the mixture as the sole source of protein for a period of thirty days This child has since received the mixture for an additional seventy days with excellent results

a subsequent trial has shown that lysine does result in some further growth improvement as well as greater feed efficiency

TABLE IV

EXAMPLES OF GROWTH IN RATS FED INCAP VEGETABLE MIXTURE 8[1]

Trial	Mixture	Feed Efficiency[2]	Initial Weight g	Final Weight g
1A	8	2 55	54	238
1B	Modified 8[3]	2 58	54	250
1C	8 with 9% skim milk[4]	2 55	54	237
1D	8 with 14 3% skim milk[4]	2 50	54	264
2A	8	2 71	44	223
2B	8 + 0 45% lysine	2 14	44	230
2C	Modified 8[3]	2 08	44	233
2D	Modified 8[3] + 0 45% lysine	1 90	44	232
2E	Modified 8[3] with 9% skim milk[4]	2 29	44	236
2F	Modified 8[3] with 9% skim milk[4] + 0 45% lysine	1 98	44	235

[1] Trials 1A to 1D and 2A to 2F were carried out with 12 rats per group 8 weeks with 25 2% protein
[2] Feed efficiency grams feed consumed per gram of weight gained
[3] Cottonseed meal replaced by additional sesame
[4] Substituted for corn masa

Work with Chicks

Baby chicks, three days of age, were fed the mixture as the sole source of protein in eight trials employing 664 chicks in 40 groups Representative data from these trials are shown in Table V Fair growth was obtained with the mixture alone and this was improved slightly by substituting whole ground corn, which increased palatability for the chick over the relatively sticky lime-treated corn (Tortilla flour) It is obvious that due to the greater requirement of the chick for lysine, this amino acid must be added if good growth is to be expected When 0 2 per cent lysine was added, the growth response of chicks improved, and when 0 4 per cent was given, the growth was excellent Higher levels of lysine did not improve the growth of the chicks fed the mixture

Generalization from the Animal Trials

It was concluded from the rat and baby chick feeding experiments that the protein quality of INCAP Vegetable Mixture 8 was adequate for

sole source of protein, vitamins, and minerals and was receiving 5 g of pro tein and 150 calories per kg by the 12th hospital day Apathy, anorexia, skin lesions, and diarrhea disappeared in three weeks, but a trace of edema persisted The child has now received the mixture for a total of 28 days and is continuing to make a good clinical recovery

C A P, a 15-month-old boy, 70 cm in height, weighed 6 8 kg when admitted with moderate kwashiorkor He was placed on the vegetable mixture as the sole source of protein vitamins and minerals and was receiving 5 g of pro tein and 150 calories per kg by his eleventh hospital day Initiation of cure* was complete at the end of 3 weeks The child has now received the mixture for a total of 26 days and is making a good clinical recovery

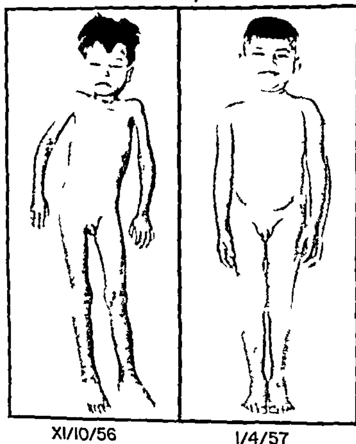


Figure 1 The photograph on the left was taken at the time of admission of E P an 8 year old boy with acute kwashiorkor and the one on the right fol lowing 7 weeks of a diet in which INCAP Vegetable Mixture 8 was the sole source of protein

We are now able to report on five additional children who have been treated with the mixture, including three who were given it from the time of their entrance to the hospital with acute kwashiorkor In each case the clinical response has been excellent and the mixture appears to be more effective than milk in reducing the tendency to diarrhea in the acute phase There is some indication that the biochemical re sponse, at least in serum protein and pseudocholinesterase was slightly slower in the three children given the mixture from the time of admis sion, but this was not reflected in the clinical progress

The photographs of one of these children (E P L) taken at the time of admission with acute kwashiorkor and following seven weeks of treat ment with only the vegetable mixture as a protein source are shown in Figure 1 The detailed case histories of the five additional children to receive the mixture are as follows

A R S , a 14 month old boy 65 cm in height, was admitted with moderate kwashiorkor and weighed 5 85 kg when his edema was lost He was placed on a mixture of 18 amino acids starch and sugar which supplied 3 0 g of pro tein and 100 calories per kg and the results will be described in more detail elsewhere At the end of 18 days the edema and skin lesions had cleared but apathy, pallor cheilitis angular stomatitis, and atrophy of the papillae of the tongue were still present Treatment with the vegetable mixture was begun and the amount increased progressively to 7 0 g of protein and 140 calories per kg The mixture furnished the only source of protein, vitamins, and minerals except for 120 cc of orange juice daily The child showed a dramatic further improvement during the next week on the vegetable mixture and was ready for discharge after receiving the vegetable mixture for 70 days At this time he appeared healthy and his weight had increased to 8 5 kg

L P L , a 5-year-old boy, 96 cm in height, was admitted with severe kwashi orkor and weighed 11 5 kg when his edema was lost He was given half-skimmed milk for five days and then changed to a diet in which the vegetable mixture furnished the only source of protein This diet provided 4 g of protein and 100 calories per kilo for 13 additional days It was then increased gradually to 5 6 g of protein and 150 calories per kilo by the addition of tor tillas, beans and other vegetables and fruits The child did very well and weighed 17 0 kg at the end of 61 days of hospitalization

E P L an 8 year-old 106 cm tall older brother of L P L was admitted at the same time with severe kwashiorkor and also weighed 11 5 kg when his edema was lost He was placed on the vegetable mixture as the sole source of protein, vitamins, and minerals until the fifth day when 120 cc of orange juice and 2 bananas daily were added to the diet By the fourth day after ad mission he was consuming 4 grams of protein and 100 calories per kilo The "initiation of cure" was judged complete 17 days after admission when tortillas beans, and other vegetables and fruits were gradually added to his diet to give a maximum of 6 0 g of protein and 180 calories per kg At this time half the protein was obtained from the vegetable mixture and half from other vegetable sources in his diet At the end of 64 days his condition was excellent and his weight had risen to 18 6 kg

J A Q , an 18 month-old boy, 68 cm in height weighed 7 8 kg when admitted with moderate kwashiorkor He was placed on the vegetable mixture as the

percentage of the nitrogen absorbed which was retained by the body was calculated, the resulting values were generally higher for the vegetable mixture

GENERAL EVALUATION OF VEGETABLE MIXTURES

The compounding of suitable vegetable mixtures is at present made difficult by two factors the uncertainty of arriving at an optimum amino acid content and balance from analytical figures alone, and the possibility that inhibitory or toxic factors may be contained in the plant ingredients selected or result from processing

AVAILABILITY OF AMINO ACIDS

The description of the amino acid pattern of a provisional reference protein has been of great help in providing a means of comparing the amino acid combinations of individual foods and food mixtures and in estimating their probable biological value As Allison has pointed out in this Symposium *(23)* there is every reason to believe that this reference pattern constitutes a useful tool, and the estimations calculated from it have thus far agreed extraordinarily well with the results of biological assays It is still a first approximation subject to further refinement, however, and it does not indicate at what point an excess of any of the essential amino acids will adversely affect the theoretical biological value calculated from the limiting amino acid

Still greater difficulty arises in extrapolating from the microbiologically determined amino acid content of vegetable proteins which have usually been subjected to drastic hydrolysis to make their amino acids available to the test organisms The availability of these same amino acids following the ordinary process of *in vivo* digestion may be different Simple *in vitro* studies following complete enzymatic digestion do not give an adequate answer either, since the relative rate of release of the amino acids has an important effect on their utilization for protein synthesis *(24)* An interesting example of this is the recent finding that the better growth observed in rats fed lime-treated corn rather than raw corn *(25 26)* may be due to the fact that the zein, which is an extremely poor quality protein in comparison to the other corn proteins, is made selectively less available by the lime treatment *(27)* The effect of cooking on the easier release of amino acids with *in vivo* digestions is a more familiar illustration Furthermore, food processing may either decrease or increase protein value

It is apparent from even this brief discussion that, however useful calculations of the potential nutritive value of vegetable mixtures may be their validity can be determined only by careful biological trials which are relatively costly and time consuming The importance of support for this vital phase of the world-wide development of suitable vegetable mixtures for human feeding is now beginning to be recognized

Metabolic Balance Studies in Children

Studies of the absorption and retention of nitrogen in children recovering from kwashiorkor on a diet in which milk was the sole or major source of protein, have been previously reported and the technique de scribed *(22)* Following the last two of these trials, the children were gradually changed over to a diet in which the vegetable mixture furnished the sole source of protein at an intake of protein and calories per kilo equivalent to that of the previous milk diet They were then studied metabolically for an additional five-day period The results are shown in the first two cases in Table VI together with the results of three additional trials in which the procedure differed in that the metabolic period on a milk diet followed rather than preceded the trial with the vegetable mixture The relative amount of nitrogen absorbed was

TABLE VI

NITROGEN BALANCE RESULTS IN CHILDREN RECOVERING FROM KWASHIORKOR

(5 day trials with 3 7 days adjustment in between)

	Weight kg	Protein/ kg	Calcium/ kg	Milk % Abs	Milk % Ret	INCAP Vegetable Mixture 8 % Abs	INCAP Vegetable Mixture 8 % Ret
	7 5	2 4	102	87	0	73	11
	11 2	2 6	106	74	23	78	23
*	9 9	3 8	110	90	15	74	12
*	9 5	2 9	101	74	11	71	14
*	11 6	2 8	108	80	34	68	23
Aver	9 9	2 9	105	81	17	73	17

* Trial with Vegetable Mixture preceded that with milk

usually less with the vegetable mixture than with the milk, and the average absorption for the five trials was 73 per cent with the mixture and 81 per cent with milk

The interpretation of the average retention requires caution because of the great variation in retention from one case to another This is an inevitable biological consequence of the differing degree of recovery and hence of repletion of depleted nitrogen stores When individual cases are examined, the retention was approximately equal in three trials, better with milk in one and with vegetable protein in the other From these initial trials it appears that the protein of the vegetable mixture once absorbed is as well utilized as that of milk When the

would appear that amino acid supplementation on the basis of our present knowledge is still a hazardous procedure

On the other hand it is entirely possible that, given sufficient knowledge, it will be found practical to improve the protein quality of the diets of underdeveloped areas with suitable combinations of those essential amino acids in which they are deficient Studies of the practicality of such a procedure are urgently needed INCAP has recently begun a series of studies in which vegetable proteins and vegetable-protein mixtures are to be brought to the theoretically desirable amino acid proportions suggested by the amino acid pattern of the provisional reference protein This is done by systematic addition of synthetic amino acids, and the nitrogen retention with each combination is observed and compared with that of an equal quantity of milk protein By adding a small amount of glycine or glutamic acid to the basal diet, the amino acid substitutions can be made without changing the proportions of other ingredients or altering the total amount of nitrogen fed Since differences in the amino acid supplements did not affect the appetite of the children in the study and the great bulk of the diet remained unchanged, a one-day adjustment period between three-day trials was considered sufficient

Because of its importance in the basal diet of Central America and because the biological value of its protein is known to be relatively poor, lime-treated corn, fed as corn *masa* or as *tortilla* was employed in the first two such trials The relation between the amino acid content of corn masa as compared with the provisional amino acid pattern is shown in Figure 2 It will be seen that the limiting amino acid de-

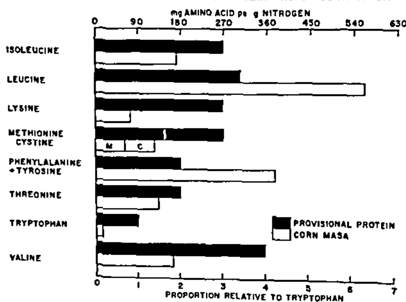


Figure 2

PROBLEM OF TOXIC FACTORS

As the search is pursued for suitable ingredients for the locally produced vegetable protein combinations, it is inevitable that many plants will be suggested for which there is no long background of either laboratory study or human use There are so many examples of toxic or interfering substances in plants that each ingredient must be carefully tested before it is assumed that it can be safely fed to human beings The necessity of destroying the enzymes soyan and trypsin inhibitor in soybean is well known, but the violent toxicity of meal made from aceituno *(Simarouba glauca)* which would otherwise be an excellent protein source, is an equally striking example *(28)* The binding of phosphorus by phytins, calcium by oxalic acid, the cyanide-containing goitrogenic substances in plants of the family Brassicae, and the toxic effect of the gossypol in cottonseed-oil meal, all illustrate the need for caution in recommending any new food mixture for human consumption To the natural hazards must be added the danger of using toxic solvents for fat extraction and the alteration of protein quality that can come about with improper processing Persons wishing to develop mixtures for human feeding along the lines of the vegetable mixture described in this report are urged to study the discussion and statement of principles laid down by the Conference on Protein Requirements and their Fulfillment in Practice held in Princeton New Jersey, in June 1955 *(29)*

AMINO ACID SUPPLEMENTATION

As the large-scale commercial preparation of certain of the key essential amino acids at a low cost becomes practical, the question is increasingly raised as to the value for underdeveloped areas of supplementing predominantly vegetable protein diets, deficient in several of the amino acids in a manner analogous to the now well-accepted enrichment program for wheat flour, corn meal and rice Much has already been written on this subject and tentative proposals have been made *(30, 31)* The situation however, is not truly comparable to the enrichment of food with vitamins Within the maximum conceivable range of enrichment with vitamins an excess of one or more vitamins has no significant effect on the utilization of the others In the case of the amino acids, however it is abundantly clear that an excess of an amino acid may in some circumstances have as devastating an effect on the biological value of the protein as a relative deficiency *(32)*

This would indicate that a great deal more must be known about the relative interaction of individual amino acids before the amino acid enrichment of foods can be seriously considered Furthermore in using the provisional amino acid pattern to determine how much of a given amino acid may be required to provide an optimum level the uncertainty of extrapolating from analytical values to biological availability in the human being again becomes a formidable obstacle With the increasing evidence that the proportion of amino acids in a diet, i e , the amino acid pattern, is more important than the absolute amounts it

arise as soon as amino acid supplementation is undertaken Regardless of the explanation, methionine addition in both cases had the unexpected effect of decreasing rather than increasing nitrogen retention

CONCLUSIONS

The results obtained thus far in the development and testing of INCAP Vegetable Mixture 8 show that it is practical to develop a low-cost all-vegetable mixture from locally available ingredients for the supplementary and mixed feeding of infants and children in underdeveloped areas It is a happy circumstance that the biological value of the protein of this Mixture 8 appears to be so closely equivalent to that of milk

Nevertheless, such a mixture need not be equivalent in biological value to milk to have an important place in the prevention of protein malnutrition, nor need it be the best possible formula from a nutritional viewpoint, but it must be effective as well as inexpensive, palatable, and easily transported and stored

Further improvements in the formula are obviously possible from both a nutritional and an economic point of view, and these will be worked out as soon as trials of the present mixture are completed A number of obvious modifications should be tested to adapt this formula to other underdeveloped areas where the availability and cost of local ingredients are different Substitution of all or part of the corn for sorghum or rice may be envisaged The use of rice instead of corn would theoretically improve its protein value and make it more palatable to rice-eating people With the availability of low-gossypol cottonseed meal, the proportions of this ingredient can probably be greatly increased or if necessary it can be left out entirely Whether or not the supplementation of diets with synthetic amino acids will prove practical and desirable can only be determined by fundamental studies to obtain data not now available

All these developments may be expected in the future in those areas which are in greatest need of them As described elsewhere in this *Symposium (33)* the current programs of WHO, FAO, and UNICEF, assisted by funds from the Rockefeller Foundation and the National Research Council will play a decisive part in stimulating the necessary scientific investigations and practical applications

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ficiencies are tryptophan, lysine, methionine, valine, and isoleucine in this order

The results of adding the first three of these amino acids to corn masa are shown in Figure 3 When the children were switched from milk to an isoprotelc diet consisting entirely of lime-treated corn supplemented by vitamins and minerals and with added calories in the form

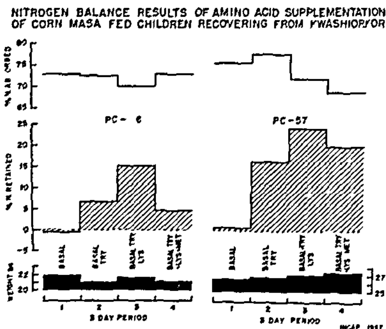


Figure 3

of sugar, starch, and margarine, the nitrogen retention dropped sharply as expected When tryptophan and lysine were added successively to bring the total amino acid content to that of the provisional protein, significant increments in the nitrogen retention were observed When methionine was added, however, the nitrogen retention dropped instead of rising further

At this point, one of the children developed chicken pox and the balance studies had to be suspended, but the other showed no improvement with valine, not until isoleucine was added and a more favorable leucine isoleucine ratio restored did recovery occur It seems possible that the amount of added methionine had an adverse effect of some kind on the leucine/isoleucine ratio and that if isoleucine had been added first, the results might have been different Only further trials can elucidate this point

These very preliminary results with amino acid supplementation are cited only to point out that the nitrogen balance method of evaluating amino acid additions seems to be an effective one to apply to the problem at hand and also to illustrate the problems of imbalance which

4

PROTEIN MALNUTRITION AS A WORLD PROBLEM

W H Sebrell, Jr , and D B Hand,
Williams-Waterman Fund for the
Combat of Dietary Diseases

The rapid development of our knowledge of the nutritive needs of man and the recognition of the importance of good nutrition to health has led in recent years to an increasing appreciation of the relation of our food supply to our well-being There are still only a relatively few people in the world however who understand that the food supply must do more than satisfy hunger if normal child development is to take place and health is to be maintained The world's agriculture is still mainly concerned with crop yields in bushels or tons per acre and with price per unit of weight rather than with nutritional needs and nutritive values Citrus fruits and tomatoes are neither produced nor sold on the basis of their vitamin-C content The only food in which price is related to protein content is wheat flour but even this is not because of nutritive value but because the protein content happens to be related to desirable dough characteristics

The identification of the vitamins and the recognition of the widespread ill health caused by their lack, has focused medical and public health attention on the vitamin-deficiency diseases such as scurvy rickets beriberi and pellagra for many years The chemical identification and synthesis of most of the vitamins their availability at low cost, and the practicability of adding them to foods together with education about the need for foods of good vitamin content has resulted in a great decrease in the prevalence of vitamin-deficiency diseases in many parts of the world Practical means are at hand to eliminate them as public-health problems For example, it is entirely feasible to eradicate beriberi by adding thiamin to the rice of Asia With the decreasing importance of these diseases as research problems has come the recognition that kwashiorkor or protein malnutrition in children is now the nutritional problem of greatest world-wide importance

AMINO ACID DEFICIENCIES

The classic studies of Rose *(1)* which showed that the young adult requires eight essential amino acids to maintain nitrogen balance opened the door to a better understanding of man's amino acid needs just as the recognition of the vitamins years ago opened the door to

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be practical Therefore, we would like to look at the international problem of protein malnutrition in the light of why it exists and what may be done about it

KWASHIORKOR

Kwashiorkor has been known under a wide variety of names for a long time and there is a very extensive literature on the subject which we will not attempt to review here In various parts of the world at different times the condition has been known by more than fifty different names but they all refer to what is essentially the same disease Although kwashiorkor seems to be the name most widely used at the present time in Africa, Europe, and the United States, the term *sindrome policarencial infantil* is preferred in Central and South America and several other names are in use The terms *Mehlnahrschaden* and *starch dystrophy* call attention to protein deficiency by emphasizing a high carbohydrate intake which supplies calories but is deficient in protein

The condition as we know it today was first recognized in the early 1900 s with some of the earliest work being done in the Americas Over the years attention was first focused on the possibility of its being a multiple-vitamin deficiency disease, although the presence of hypoproteinemia was noted and treatment with vitamin preparations under controlled conditions failed therapeutically Numerous contributions have been made to the study of the disease from many parts of the world Cofino and Klee *(3)* in San Salvador in 1938 seem to have been among the first to stress the use of fermented skim milk or buttermilk in treatment Since about 1944 the importance of protein in the syndrome has received increasing attention and although multiple-vitamin deficiencies parasites, infection dehydration, liver impairment, enzyme lack, salt imbalance, and possibly other factors may be involved, the view today is that the underlying defect is protein or amino acid deficiency that dry skim milk is an effective treatment, and that, if the child has adequate protein, the disease does not occur The excellent monographs of Brock and Autret *(4)* in 1952 on kwashiorkor in Africa and of Autret and Behar *(5)* in 1954 on the condition in Central America summarize much of our information to that time

WORLD FOOD PRODUCTION

The modern world s nutrition is largely based on a few staple foods These vary in different parts of the world but the principal ones are wheat rice corn, millet and cassava Cassava ranks second only to rice in the amount consumed in the underdeveloped areas of the world as a staple food and source of calories and it is without doubt the poorest in nutritive quality It is widely used either as a staple food or as a supplement to cereals in South America Africa and Asia If the consumption of this product could be replaced with another staple prod-

another wide area of knowledge There is still much to be learned about man's amino acid requirements and the research of the next few years will add much new knowledge to that subject Enough is now known, however, to recognize the extent of kwashiorkor as a health problem and to take measures which can prevent a multitude of deaths and much sickness and disability Some possibilities for a solution to the prob lem of supplying the population of the world with adequate amino acids as well as adequate vitamins and other nutrients are now becoming apparent

Kwashiorkor has so captured medical and scientific attention in the past few years that there is a tendency to assume that kwashiorkor and protein or amino acid deficiency are completely synonymous This is not the case Kwashiorkor is only one form of protein malnutrition Famine edema is another form Methionine deficiency plays a role in cirrhosis of the liver tryptophan deficiency is involved in pellagra and, as our knowledge becomes more complete, it is probable that we will be able to pin-point many more specific amino acid functions in disease Nevertheless kwashiorkor because of its severity and its effects on so many children in so many parts of the world, is the form of amino acid deficiency that is most important from a health point of view Our knowledge is so recent and fragmentary that some of you may be questioning whether kwashiorkor should be regarded as an amino acid de ficiency or not This is based on the work of Brock and his associates *(2)*, who have obtained initiation of cure with a mixture of synthetic amino acids This work seems to eliminate the possibility of another primary cause although the etiology is still not completely identified For our purposes here we can regard it as protein malnutrition since dried skim milk is an effective treatment From a practical point of view, we must at this time look at the problem as one of prevention through supplying foods containing protein of good biological quality designed to meet the essential amino acid needs of small children This should be possible even though we still do not know the small child s requirements for the essential amino acids

As an international problem protein malnutrition is one of those conditions in which the remedy is very easy to state but exceedingly difficult to apply We would not have any protein malnutrition problem if all the children in the world had plenty of milk, either fresh or dry skimmed Therefore our first concern is to make use of all the milk available and to push the development of milk production and conservation wherever this is possible If we do this to the utmost we still will not have made an appreciable mark on the occurrence of protein malnutrition, because the areas in which it is most prevalent are those where milk is not available and where there is little prospect of its becoming available through local resources for many years The continued gift of surplus dried skim milk from the United States is only a temporary help any permanent solution under present world conditions must rest within the resources of the areas concerned A study of this problem reveals that there are relatively few possible answers that appear to

TABLE II

PRODUCTION OF LOW PROTEIN CROPS IN KEY COUNTRIES *(7)*
1000 metric tons

	Mexico	Brazil	Nigeria	Egypt	Turkey	India	Indonesia	Philippines
Cassava		14 493	10 722			1 278	9 443	290
Sweet potatoes and yams	71	939	9 998	52		1 272	2 038	770
Potatoes	150	815		225	1 000	1 626	56	7
Sugar cane	12 447	40 302		4 215	1 165*	55 522	6 391	12 558
Sugar	1 094	2 425		319	195	4 454	968	1 304
Bananas	205	3 964	74	37		1 879		208

Sugar beets

foods in these same areas *(7)* From food-production data some interesting possibilities can be developed that may shed light on why protein malnutrition is more prevalent in some areas than in others, why kwashiorkor affects children and why protein malnutrition may not be so evident in adults

We can attempt to answer the question are any of these staple foods capable of meeting protein requirements without supplementation?

If we assume that the protein requirement of a 60-kg man is 21 grams of an ideal protein with a biological value of 100 and allow a 50 per cent margin for safety, the minimum daily requirement of ideal protein would be 31 5 grams Actual food requirement then would vary, depending on the biological value of the protein in that food The limiting factor on the total amount of food is the number of calories and the volume of food which the individual can obtain These calculations are shown in Table III *(8 9)* arbitrarily assuming a 2000-calorie intake as the practical limitation

TABLE III

PROTEIN INADEQUACY OF STAPLE FOODS FOR ADULTS
Based on adult male weighing 60 kilograms requiring 31 5 grams daily of protein with biological value of 100 *(8 9)*

Food	Protein Content g/100 g	Calories per 100 g	Estimated Protein Score	Daily Protein Requirements grams	Protein accompanying 2000 cal intake
Rice *	7	363	72	44	39
Maize	9	355	42	75	48
Wheat* (whole grain)	12	332	59	53	72
Millet and sorghum *	10 5	331	68	46	63
Cassava	1 1	131	22	143	17

Average or approximate figures

uct of satisfactory protein value it would undoubtedly be a great factor in preventing protein malnutrition

Table I shows the production of five staple foods in eight selected countries (7) These countries have been selected because data are

TABLE I

PRODUCTION OF STAPLE FOOD CROPS IN SELECTED COUNTRIES
Production data for 1954 or latest year reported — 1000 metric tons (7)

Country	Wheat	Maize	Millet and sorghum	Rice	Cassava
Mexico	830	4 000		162	
Brazil	871	6 096	-	3 266	14 493
Nigeria		755	2 888	250	10 722
Egypt	1 729	1 752	549	1 118	
Turkey	5 010	914	88	176	
India	7 999	2 991	17 120	38 894	1 278
Indonesia		2 668		11 793	9 443
Philippines		770		3 203	290

available from them and they represent important parts of the world Nigeria from Africa India and all Indonesia from Asia Turkey and Egypt from the Middle East and Brazil and Mexico from the Americas All the data presented in this paper will relate to these countries as examples and the United States figures sometimes will be included for comparative purposes The selection of these countries in no way indicates any specific problem in protein malnutrition in that country They are cited as illustrations of the protein problem as it affects the world

The figures have not been put on a per capita basis because for our purposes here such data are misleading For example in a country in which the cassava production is high and the rice and maize production also relatively high it is not likely that a per capita figure for cassava production will have any relation to the amount actually consumed by an individual Those who eat cassava probably eat a lot of it and those who eat corn probably eat a lot of it and realistic figures are not available We have therefore just given total production figures which indicate that some elements of the population of that country are probably eating a lot of the product

These countries have also been selected on the basis that their population exceeds 20 million persons and production of at least one of the staple foods exceeds 1 million metric tons Therefore it is safe to assume that in these areas large numbers of people are subsisting on a basic diet of one of these principal food crops

Table II also shows the large production of some other low protein

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Sugar	1 094	2 425		319	195	4 454	968	1 304
Bananas	205	3 964	74	37		1 879		208

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*Average or approximate figures

Thus it can be seen that it is theoretically possible for an adult man under these limitations to meet his protein needs from millet or wheat while from cassava only approximately 10 per cent of the protein need could be met

If we take the same problem for children, the situation is quite different Table IV *(9 10)* gives the protein and calorie requirements for

TABLE IV

DAILY PROTEIN AND CALORIE REQUIREMENTS FOR CHILDREN *(9 10)*

Years	Requirements* for protein gm per kg body weight	Body* weight Kg	Minimum protein requirement gm	Daily calorie requirements	Minimum protein requirement gm per 100 cal
0	2 1	3 4	7 1	300	24
9/12	1 4	8 4	11 8	650	18
2	1 2	12 3	14 8	1100	13
4	0 9	16 4	14 8	1400	10
6	0 75	20 5	15 4	1600	9
8	0 7	24 8	17 4	1800	9
10	0 7	30 5	21 3	2000	11
12	0 70	36 4	25 5	2100	12
15	0 8	50 5	40 4	2300	18
16	0 7				
17	0 55				
18	0 45				
21	0 35				

*Protein requirements are minimum average values for proteins of biological value of 100 To get safe population requirements add 50% and correct for biological value of protein in diet

children, and it is seen that the ratio of protein to calorie requirement is relatively high at ages through four years and during adolescence, a ratio which may be interpreted to mean that calories must be supplied by foods richer in protein than is the case for adults

If we now calculate the protein requirements for children on the basis of the same staple foods we used in the calculations for adults in Table V *(10)*, it is evident that all these foods must be supplemented for young children Wheat and millet still meet the need above age two but cassava is practically useless

Now our next question is what are the available food possibilities for improving the quantity and quality of the protein of the staple foods? First, let us look at the production of high-quality protein food in our selected countries Table VI *(9 11)* shows a number of interesting things First, Nigeria has practically no animal protein resource of

TABLE V

PROTEIN INADEQUACY OF STAPLE FOODS FOR YOUNG CHILDREN *(10)*

Age in years	0	9/12	2	4	6
Daily calorie requirement	300	650	1100	1400	1600
Rice					
Actual protein requirement*	15	25	31	31	32
Protein accompanying* calorie requirement	6	12	21	27	31
Maize					
Actual protein requirement	25	42	53	53	55
Protein accompanying calorie requirement	8	17	28	36	41
Wheat					
Actual protein requirement	18	30	38	38	39
Protein accompanying calorie requirement	11	23	40	51	58
Millet sorghum					
Actual protein requirement	16	26	33	33	34
Protein accompanying calorie requirement	10	21	35	44	54
Cassava					
Actual protein requirement	48	80	101	101	105
Protein accompanying calorie requirement	3	6	9	12	14

*Calculations based on data in Table IV

any kind Secondly, Indonesia, including the Philippines is very limited in animal protein although fish is relatively important Turkey and Mexico present some similarity in their problems Increases in milk may be feasible and important in India and Brazil On the whole, however our problem of protein malnutrition in underdeveloped countries

TABLE VI

PRODUCTION OF ANIMAL PRODUCTS IN SELECTED COUNTRIES *(7 11)*
1000 metric tons

	Brazil	Mexico	Nigeria	Egypt	Turkey	India	Indonesia	Philippines
Milk*	3 215	1 760	14	904	2 563	19 318	15	2
Meat†	1 611	211		195	121		253	60
Eggs *	237	120		27	56	55	132 5	52 5
Fish	172 0	67 3	42 0	63 4	111 5	839 0	651 5	385 3

*Cow goat sheep and buffalo milk
†Includes beef veal pork mutton and lamb (excludes poultry)
**Hens eggs

Thus it can be seen that it is theoretically possible for an adult man under these limitations to meet his protein needs from millet or wheat while from cassava only approximately 10 per cent of the protein need could be met

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9/12	1 4	8 4	11 8	650	18
2	1 2	12 3	14 8	1100	13
4	0 9	16 4	14 8	1400	10
6	0 75	20 5	15 4	1600	9
8	0 7	24 8	17 4	1800	9
10	0 7	30 5	21 3	2000	11
12	0 70	36 4	25 5	2100	12
15	0 8	50 5	40 4	2300	18
16	0 7				
17	0 55				
18	0 45				
21	0 35				

*Protein requirements are minimum average values for proteins of biological value of 100 To get safe population requirements add 50% and correct for biological value of protein in diet

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TABLE VIII

AVAILABLE PROTEINS FROM SUPPLEMENTARY SOURCES *(7 11)*
Grams per capita per day

	Mexico	Brazil	Nigeria	Egypt	Turkey	India	Indonesia	Philippines
Meat	2	8		2	1	-	1	1
Milk	7	6		4	12	5	-	-
Fish	1	1		1	1	1	2	5
Pulses	11	16	-	8	7	17		1
Soybeans	-	2		-		-	5	-
Peanuts	2	3	19			7	4	
Se ame	2	-		1	1	1	-	-
Cottonseed	13	8	1	17	6	2		-
Copra		-			-	-	1	6

Protein compositions used in calculations milk 4%, pulses 22% soybeans 38%, pea nuts 26% sesame 19%, cottonseed 21%, and copra 5% meat 10% and fish 10%

Table IX *(8)* there is presented an analysis of some samples of these types of foods on the basis of protein in relation to calorie content This shows that the pulses are between the animal products and the oil seeds in protein per 1000 calories Now let us look at the quality of the protein available in terms of essential amino acids *(6)* In Table X *(12)* we can see that the problem of getting a product as good as dried skim milk in sufficient quantity probably has different answers in different parts of the world In all likelihood the answer resides in adding suitable supplementary foods, either singly or in mixtures, to the diet In some areas of the world it may be fish flour, in others, pulses, in others, oil seeds, or preparations and mixtures made from these products

Our research effort today is concentrated on finding an adequate food preparation that is safe, easy to produce, and will maintain health and growth in children After a product is theoretically satisfactory it must be proved to be practical by suitable tests on children After this problem is solved, and it should be solved in a relatively short period of time now, the really difficult problems are then before us These are how to get the desired foods produced in adequate amounts where they are needed, and how to get them accepted by the people who need them This involves some basic considerations which cannot be ignored in a practical approach to the problem

After we know beyond question what the correct products are and the needed amounts the next step would seem to be to teach people to produce and use these products This will not be easy In Table XI *(7 13 14)* we have listed the populations of our selected countries on the basis of inhabitants per hectare of cultivated land the consumption

is not likely to be solved from animal sources Therefore, let us look at the production of protein-rich foods of plant origin in these same areas as in Table VII *(7)* If we look first at Nigeria, we see that the

TABLE VII

PRODUCTION OF PROTEIN RICH PLANT FOODS IN SELECTED COUNTRIES *(7)*
1000 metric tons

	Mexico	Brazil	Nigeria	Egypt	Turkey	India	Indonesia	Philippines
Dry beans	400	1 464		2	105	1 197	-	40
Dry peas	4	-			1	588	-	1
Broad beans	22	42		239	37		-	
Chick peas	92			6	75	4 852		
Lentils	3			60	64	213	-	
Pigeon pea		-				1 856		
Other pulses		-				1 908		
Soybeans		113			4		400	
Peanuts	74	219	790	24	15	3 884	448	18
Sesame seed	92	5	14	15	48	601	2	-
Rape seed	6	-		-	2	977	-	
Cottonseed	668	835	70	673	260	1 528	1	1
Sunflower seed					120		-	
Copra	75		7			180	760	942

only one in any quantity is peanuts We also see that India has a large production of a variety of foods of this type especially the pulses These foods offer a possible answer here Rather surprisingly Indonesia is the only one of our sample countries that shows soy beans as an important crop Here the possibilities would seem to lie in soy beans and peanuts

Cottonseed is important in Mexico Brazil Egypt and India and copra has possibilities for the Philippines and the rest of Indonesia

Table VIII *(7 11)* breaks down the available protein from supplementary sources on a per capita basis at present production levels We have assumed a protein composition for these calculations This *also shows* that pulses are important for Mexico Brazil and India It shows also the surprising importance of milk in Turkey It again points up the great importance of peanuts in Nigeria and of cottonseed in Mexico and Egypt

NEW DIETARY PREPARATIONS

Therefore, it would *appear that the answer to the world s* protein supply must be sought in animal products pulses and oil seeds In

TABLE X

ESSENTIAL AMINO ACIDS IN SELECTED FOODS *(6)*

Percentage of *crude protein*

Product	Crude Protein G per 100 g product	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Dried skim milk	33 44	3 23	2 33	5 98	9 23	6 31	2 72	5 17	4 87	1 43	6 84
Herring meal	72 25	5 72	2 37	5 74	8 15	8 19	2 92	4 04	4 51	1 55	6 05
Sardine meal	68 25	5 89	2 64	5 10	7 81	8 58	2 99	4 28	4 45	1 30	5 96
Corn yellow	8 05	4 60	2 73	4 10	11 80	3 35	2 24	5 22	3 85	1 17	5 34
Rice Texas Patna, Polished	7 88	8 25	2 41	4 44	7 99	3 30	2 54	4 57	3 43	1 59	6 34
Wheat, Seabreeze	18 50	5 46	2 70	3 84	6 11	2 70	1 24	4 86	2 86	1 21	4 61
Millet Hog (proso)	12 14	2 89	1 90	4 04	10 13	2 06	2 47	4 86	3 63	1 38	5 12
Copra meal	22 22	11 43	1 71	4 00	6 30	3 06	1 59	4 23	3 78	0 94	5 49
Cottonseed flour (Proflo)	54 07	11 30	2 57	3 94	6 23	4 16	1 61	5 20	3 36	1 55	4 84
Peanut flour	61 00	11 26	2 16	4 33	6 74	3 20	1 02	5 08	2 67	1 28	4 64
Sesame meal	46 08	11 91	2 21	4 27	6 92	2 76	2 65	4 73	3 64	1 91	5 06
Soybean meal	45 29	7 46	2 49	5 50	7 69	6 17	1 39	4 86	4 03	1 69	5 40
Black eyed peas	22 31	6 41	2 82	4 62	7 22	6 59	1 52	5 38	3 58	1 03	5 29
Cow peas mixed	24 14	5 27	3 03	4 72	7 17	6 30	1 16	5 18	4 31	1 33	5 56
Mexican Pinto beans	22 58	6 85	2 83	5 05	4 92	7 08	1 15	5 31	4 82	1 42	5 54
Soybeans Laredo	35 02	8 84	2 29	4 68	5 80	6 47	1 37	4 77	3 68	1 60	5 39

TABLE IX

PROTEIN AND CALORIE CONTENT OF SELECTED SUPPLEMENTAL FOODS *(8)*

	Water per 100 gm	Cal per 100 gm	Protein per 100 gm	Protein per 1000 cal
Animal products				
Cow's milk, fresh	87 0	66	3 7	56
Cow s milk, dried skim	3 5	362	35 6	8*
Buffalo milk	82 2	106	4 7	44
Eggs (as purchased)	66	144	11 4	80
Beef thin (as purchased)	64	164	15 2	9*
Mutton, thin (as purchased)	45 7	142	11 8	83
Pork thin (as purchased)	41 0	311	11 6	37
Sardines (as purchased)	47 1	175	21 1	121
Codfish (as purchased)	39 6	36	7 9	219
Pulses				
Dry beans *(Phaseolus vulgaris)*	12 2	336	23 1	68
Dry peas *(Pisum sativum)*	11 6	339	23 8	70
Broad beans *(Vicia faba)*	12 2	338	25 4	75
Chick peas *(Cicer arietinum)*	10 6	359	20 8	57
Lentils *(Lens culinaris)*	11 2	337	25 0	74
Pigeon peas *(Cajanus cajan)*	13 1	333	21 9	65
Cow peas *(Vigna sinensis)*	10 8	342	22 9	66
Field beans *(Dolichos lablab)*	11 8	338	22 2	65
Mung beans *(Phaseolus aureus)*	11 0	339	24 4	71
Oil seeds				
Soybean seeds (fat 18 1%)	7 5	331	34 9	105
Soybean flour (fat 6 6%)	8 2	244	38 4	157
Peanuts as purchased in shell (fat 30 4%)	2 8	389	18 6	48
Peanut flour (fat 6 9%)	2 3	327	42 8	131
Sesame seed (fat 51 1%)	5 8	568	19 3	34
Sesame flour (fat 5 7%)	10 9	257	36 7	143

of power, and the per cent of illiteracy, since these are some of the basic elements that enter into food production It is seen immediately that in Egypt and Indonesia, especially there is already a population problem, while in Nigeria, India, Indonesia including the Philippines, there is a lack of power and therefore lack of industrial development A large part of the problem of education is not only teaching the individual that he needs certain foods but in educating the agricultural planners that this is the best way to use a limited land area — an area which is crowded already in some places and where there are economic pressures to raise other crops for a variety of reasons of national importance — *and finally, educating* the individual *where the illiteracy* is above 50 per cent of the population, is going to require some special methods of teaching as well as some new incentives to learn and to apply knowledge

It seems quite clear that protein malnutrition in the world is more than a scientific problem and that it involves aspects of a socioeconomic nature Here is a great opportunity for international cooperation

The United Nations Children's Fund has a particular interest in the welfare of children and mothers and occupies an unusual position among United Nations agencies in that it is supported by contributions from both governments and private individuals It is in essence a relief agency for the distribution of contributions to help children in needy areas This agency, generally referred to as UNICEF, receives technical advice on matters related to food from the Food and Agricultural Organization in Rome and advice on health and medical problems from the World Health Organization in Geneva These three agencies working in close cooperation, can bring to bear on the problem of protein malnutrition talent and resources that never have been available before

The Rockefeller Foundation has generously made available $250 000 to start research work on finding the proper mixtures of foods that will solve the problem in the different areas When these products have been determined the need will arise for an extensive and well-planned program to stimulate production and education in their use

If we succeed in preventing kwashiorkor, we will not only save the lives of literally millions of children we will also be producing healthier young people, better able to work and produce and carry more of their share of the world's responsibilities

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TABLE XI

GENERAL COMPARISONS OF SELECTED COUNTRIES *(7 12 13 14)*

Country	Inhabitants per hectare of cultivated land (population 1954)	Per capita power consumption in coal equivalents (metric tons 1954)	Per cent illiteracy (Ages 10 and over) (1945 1954)	Farm wages per day (in US$) (1952)	Per capita National Income (in US$) (1952)
United States	1 2	7 62	2 5	5 30	1 845
Mexico	1 5	0 65		0 53	221
Brazil	2 8	0 34	51		143
Nigeria	1 5	0 04			
Egypt	9 2	0 24	74		
Turkey	1 0	0 34	70	7 50 (per week)	196
India	2 4	0 11	82	0 28	56
Indonesia	7 3	0 09		-	90
Philippines	3 6	0 11	38	0 97	166

sate itself was obtained, but which fell considerably short of Madden's mixture Fractionation of the acid-hydrolyzed casein was therefore undertaken The monoaminomonocarboxylic acids were obtained by butanol extraction, and the basic amino acids by adsorption on a sulfonic acid ion exchange resin in the ammonium form Glutamic and aspartic acid were discarded in the effluent from the resin column These two fractions combined in the proper proportions and, fortified with DL-tryptophan, gave a preparation which was tolerated at much higher infusion rates than Madden's VUJ mixture It was soon found that the reason for the superiority of the preparation was that it consisted almost entirely of the L-amino acids, whereas the VUJ mixture contained about 25 per cent of acids on the D- form The new preparation was called the VUJN mixture, the N indicating its greater natural amino acid content Subsequent studies with pure compounds showed the D-forms to be much more emetogenic and that the ameliorating effect of glycine observed by Madden was due to its property of reducing the nausea-producing effect of the D-amino acids *(5)*

It was soon found that a second method gave higher yields of an equally satisfactory product An anion exchanger was used to remove simultaneously the hydrolyzing and dicarboxylic acids All the remaining amino acids were then adsorbed on a sulfonic acid cation exchanger in the hydrogen form All unidentified emetogenic substances were discarded in the effluent The amino acids were eluted with ammonia and supplemented with tryptophan and glycine Table I shows the rates at which the various preparations tested can be infused in the dog without producing vomiting

Large quantities of VUJN solutions were made and tested clinically No more side reactions were observed than with normal saline, even

TABLE I

RATES OF INFUSION OF AMINO ACID MIXTURES

Preparation	Rate of infusion in mg of N 1 kg/min required to cause vomiting in the dog when a total of 210 mg of N/kg is infused
Acid hydrolyzed casein	1 2 - 2 0
Autoclaved acid hydrolyzed casein	3 0
Acid hydrolyzed casein minus dicarboxylic acids	3 0 4 0
Enzymatically hydrolyzed casein	4 0
VUJ mixture (crystalline amino acids)	6 0
VUJN first process	> 20
VUJN second process	> 20

5

AMINO ACID MIXTURES IN HUMAN NUTRITION

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J F Brock and J D L Hansen, University of Cape Town
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Some years ago it became clear that further studies of human nutrition would be facilitated by the use of amino acids and their mixtures Merck & Company, therefore, in 1940 undertood their production for clinical investigation As a criterion of purity, the method of solubility analysis was adopted as the best one available *(1)* Methods were developed for producing all the amino acids with a purity of 99 per cent or better with the exception of L-tryptophan and L-arginine hydrochloride Since a racemic tryptophan of very high purity is readily prepared, it is believed that the solubility analysis method failed in the case of L-tryptophan because the acid has a greater solubility and hence a greater instability than the others With L-arginine hydrochloride a purity of greater than 97 per cent by the solubility method has not yet been attained and the reason remains obscure Because the solubility method is tedious and time consuming it is used only in the evaluation of the primary standards, the microbial method being used in replicate as a secondary assay method Since 1940 the cost of producing pure amino acids has been considerably reduced so that clinical trials with amino acid mixtures are no longer prohibitive

AMINO ACID SOLUTIONS FOR PARENTERAL USE

Investigations concerning the possible place of amino acid mixtures in human nutrition were undertaken as soon as the acids became available Emphasis was placed upon the preparation of satisfactory amino acid solutions for parenteral use Dr Sidney Madden and his associates *(2 3 4)* then of the University of Rochester had found that a solution of essential amino acids was much better tolerated than the available protein hydrolysates and also made the important observation that glutamic and aspartic acids were extremely emetogenic He found also that glycine would increase the tolerance of the dog to the amino acid solutions he was using One of Madden s mixtures, designated as the VUY mixture, was made up in considerable quantity and tested clinically

Because of the clinical acceptability of VUJ mixture its duplication by modification of a protein hydrolysate was undertaken The dicarboxylic acids were removed from acid hydrolyzed casein by a variety of methods, and in every case a more acceptable product than the hydroly-

sate itself was obtained, but which fell considerably short of Madden's mixture Fractionation of the acid hydrolyzed casein was therefore undertaken The monoaminomonocarboxylic acids were obtained by butanol extraction, and the basic amino acids by adsorption on a sulfonic acid ion exchange resin in the ammonium form Glutamic and aspartic acid were discarded in the effluent from the resin column These two fractions combined in the proper proportions and, fortified with DL-tryptophan, gave a preparation which was tolerated at much higher infusion rates than Madden's VUJ mixture It was soon found that the reason for the superiority of the preparation was that it consisted almost entirely of the L-amino acids, whereas the VUJ mixture contained about 25 per cent of acids on the D- form The new preparation was called the VUJN mixture, the N indicating its greater natural amino acid content Subsequent studies with pure compounds showed the D-forms to be much more emetogenic and that the ameliorating effect of glycine observed by Madden was due to its property of reducing the nausea-producing effect of the D-amino acids *(5)*

It was soon found that a second method gave higher yields of an equally satisfactory product An anion exchanger was used to remove simultaneously the hydrolyzing and dicarboxylic acids All the remaining amino acids were then adsorbed on a sulfonic acid cation exchanger in the hydrogen form All unidentified emetogenic substances were discarded in the effluent The amino acids were eluted with ammonia and supplemented with tryptophan and glycine Table I shows the rates at which the various preparations tested can be infused in the dog without producing vomiting

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Enzymatically hydrolyzed casein	4 0
VUJ mixture (crystalline amino acids)	6 0
VUJN first process	> 20
VUJN second process	> 20

when 500 cc of a 10 per cent solution was infused in as short a time as ten minutes In addition, the loss of free amino acids in the urine at these high rates were quite low These results are reported elsewhere *(6-13)*

PHENYLALANINE-FREE CASEIN HYDROLYSATE

In 1953 the preparation of a phenylalanine free hydrolysate was undertaken for Dr Marvin Armstrong of the University of Utah Medical School Using mixtures of crystalline amino acids, he had been able to obtain indications that the metabolic disease known as *phenylketonuria* might respond to treatment with a phenylalanine-low diet In the course of the VUJN work, it was found that incomplete washing of the anion exchange column, used for the adsorption of the dicarboxylic acids, resulted in the loss of a large part of the phenylalanine — so much, in fact, that some of the preparations were found to be deficient in this essential amino acid That column was therefore used for the preparation of a phenylalanine-free hydrolysate It was soon found however, that using the high pH necessary, appreciable quantities of threonine and histidine were eliminated It became necessary then to remove the aromatic amino acids by adsorption on charcoal

Because of the key role of glutamic acid in amino acid metabolism, Dr Armstrong did not wish to eliminate this compound He had encountered difficulty however, in feeding children with hydrolysates containing high concentrations of glutamic acid In our work on the amino acid solutions we had also observed that glutamic acid, when converted to its lactam, pyrrolidonedicarboxylic acid, by autoclaving in solution, was entirely innocuous Large quantities of the lactam could be ingested orally or parenterally without producing nausea In the course of his work, Dr Armstrong also made this observation independently The following procedure for the preparation of the phenylalanine-free hydrolysate was therefore adopted Casein was hydrolyzed with mineral acid which was removed by an anion exchange resin The quantity of resin used was so adjusted that somewhat more than half the aspartic acid was eliminated although a higher percentage of glutamic acid was retained The phenylalanine was removed by charcoal and the remaining glutamic acid was converted to pyrrolidonedicarboxylic acid by autoclaving The resultant solution was spray dried and supplemented with tryptophan, tyrosine, and a mixture of minerals This mixture, the composition of which is shown in Table II was used by Dr Armstrong in feeding a number of phenylketonurics and, as has been reported *(14)* was found to be effective in the prevention of the mental deterioration which accompanies this disease

ETIOLOGY OF KWASHIORKOR

Also in 1953, one of us (J F B) visited this country after having just completed a survey of kwashiorkor in Africa under the auspices of WHO

He was struck by the fact that dried skim milk was so effective in the initiation of cure of this disease and was curious to determine which factors in the skim milk were responsible for its curative properties *(15)* Accordingly we undertook a collaborative project which is still

TABLE II

PHENYLALANINE DEFICIENT CASEIN HYDROLYSATE

L arginine	1 0%	DL tryptophan	1 0%
L histidine	1 7	L valine	6 9
L isoleucine	5 4	L phenylalanine	0 1
L leucine	12 9	L tyrosine	5 0
L lysine	9 3	L aspartic acid	2 5
D methionine	0 5	L glutamic acid	2 5
L methionine	2 3	Pyrrolidonecarboxylic acid	12 0
L threonine	5 7	Glycine	6 2

continuing Our first approach was to prepare a semipurified diet consisting of Labco vitamin free casein, glucose, a mineral mixture patterned after the mineral content of skim milk, and a vitamin mixture of all known vitamins This mixture was found to be equally as effective as skim milk *(16)* Curiously enough, its efficacy was not appreciably influenced by withdrawal of the vitamin mixture The next step was to prepare a diet in which the casein was replaced by a mixture of eighteen amino acids This mixture was patterned after casein A good many of the amino acids are readily available only in the racemic form Since it was felt necessary to double the quantities of the racemics, except in the case of DL methionine, we ended up with a diet containing 59 5 per cent amino acids This mixture was also found to be efficacious in the initiation of cure, and again elimination of the vitamins did not greatly decrease its efficacy *(17)* These results show that no unknown factors are involved in the initiation of cure of the kwashiorkor syndrome

At this point in the interest of saving time and amino acids, it was decided to take a gamble The next mixture was designed to show whether the essential amino acids were effective, not in the pattern of casein, but in a pattern based to some extent on our meager knowledge of infant requirements This mixture, in addition to the ten amino acids necessary for growth, also contained L tyrosine, which was included to spare phenylalanine, which is readily available only in the racemic form The diet formulated contained 16 per cent of this mixture, and it effected initiation of cure *(17)* When used without the vitamin mixture, however, its effectiveness was much decreased At present it is not known whether the need for vitamins is increased in a low amino acid ration or whether the increased requirement is due to their involvement in the

TABLE III

COMPOSITION OF DIETS

	C 2	A 2	A 3
Casein Labco vitamin free	38 0%		
Amino acids mixture		59 5%	16 0%
Salt mixture	9 6	9 6	9 6
Vitamins	0 2	0 2	0 2
Glucose	52 2	30 7	74 2
	100 0	100 0	100 0

conversion of the essential to nonessential amino acids We hope soon to investigate the effect of vitamin B_6, which plays such a key role in *the metabolism of amino acids*

The compositions of the diets used are shown on Table III, the com position of the amino acid mixtures in Table IV

TABLE IV

COMPOSITION OF AMINO ACID MIXTURES

	A 2	A 3
L arginine HCl	3 3%	7 8%
L histidine HCl H_2O	2 2	4 4
L isoleucine & 50% alloisoleucine	9 6	9 0
L leucine	7 1	10 1
L lysine HCl	7 0	12 6
DL methionine	2 0	4 4
DL phenylalanine	5 0	17 2
DL threonine	6 0	9 1
DL tryptophan	1 9	3 1
DL valine	8 9	6 3
DL alanine	5 0	
DL aspartic acid	10 3	
L cystine	0 6	
L glutamic acid	13 8	
Glycine	1 5	
L proline	6 4	
DL serine	5 5	
L tyrosine	3 9	16
	100 0	100 0

These mixtures were all effective in the initiation of cure from a clinical viewpoint They were not equally effective as nitrogen sources *(18)* Nitrogen balance data obtained to date are still meager but as shown in Table V they do indicate that the eighteen amino acids formula may be superior to the one containing eleven amino acids and not greatly inferior to a milk diet These experiments are now being repeated

TABLE V

NITROGEN BALANCES
(See reference 18)

Formula	Case No	Nitrogen Intake (g per kg daily)	Percentage Absorption	Nitrogen Retention (g per kg daily)	Percentage Nitrogen Retention	Balance Period (days)
18 Amino Acids	1	0 88	81	0 284	32	7
+ vitamins	2	0 955	77	0 374	38	6
18 Amino Acids no vitamins	3	0 571	85	0 26	45	7
11 Amino Acids	4	0 31	69	0 049	15	3
+ vitamins	5	0 257	74	0 073	28	4
11 Amino Acids	6	0 311	75	0 087	28	5
no vitamins	7	0 245	75	0 033	14	3
Half cream	8	0 62	84	0 311	50	7
milk	9	0 896	80	0 434	48 4	10
11 Amino Acids + vitamins + glycine	4	0 64	71	0 131	20	3

under exactly comparable conditions In using DL-methionine as equivalent to the L-isomer, we may have fallen into a pitfall You may recall a year ago at this conference, Dr Nasset *(19)* reported that the two were equivalent in rats in good nutritional state but that depleted animals were unable to utilize the D-isomer Certainly our subjects are protein depleted

From Table V it can be seen that on the average, even with amino acid mixtures in which digestion plays no part, about 25 per cent of the nitrogen is eliminated in the feces Orten *(20)* has demonstrated in a blind intestinal loop that the absorption of one amino acid may be greatly inhibited by a second It is possible that even though we supply a mixture which would give 100 per cent retention in a normal child, we are, in effect, because of poor and unequal absorption, administering an unbalanced mixture deficient in one or more of the essential amino acids By compensating for those acids the absorption of which is inhibited, we may be able to devise much more effective mixtures Such mixtures might be of value in any case of severe diarrhea in which absorption is incomplete

Having established that kwashiorkor is very likely caused by an amino acid deficiency or imbalance, we wished to demonstrate which amino acids were involved It is a well-established fact that the feeding of a mixture lacking in a single essential amino acid is often more deleterious than giving a protein-free diet Therefore, the consecutive deletion of essential amino acids from a mixture of proved efficiency seemed unlikely to yield meaningful results The most logical course of action appeared to be supplemention of the maize diet, upon which the disease developed, in such a way that the patient would receive approximately the quantity of each amino acid found in our curative mixtures Since the protein content of maize is only approximately 9 per cent, it was reasoned that more satisfactory results could be ob tained by using a high-protein concentrate of this grain In order to accomplish this we enlisted the aid of Mr E E Daggy of the Corn Products Refining Company, who kindly supplied quantities of protein fractions which were obtained in the processing of corn under very mild conditions These fractions when properly combined yielded a product containing 45 per cent protein with much the same amino acid composi tion as whole corn itself This concentrate was used in preparing our M type of formulations

The minimum requirements of the essential amino acids for initiation of cure had not yet been established The possibility existed therefore that corn meal itself supplemented with the amino acids in which it is deficient together with a source of nonspecific nitrogen, might be efficacious Accordingly African corn meal supplemented with glycine and DL-alanine has also been used as the basis for a second type of diet which we have designated as *S formulations*

As is well known, corn protein is deficient in both lysine and trypto phan In addition, Elvehjem *(21)* has recently reported that less than 20 per cent of the isoleucine of zein is available to rats These three amino acids have therefore been added to our experimental diets The composition of the M 2 and S 1 diets is shown in Table VI in compari son with the A-3 preparation which has been shown to be effective in the initiation of cure

The M-2 and S-1 diets were used in the treatment of typical kwashiorkor patients The M-2 formulation proved unpalatable to such an extent that its use was soon discontinued However with those subjects who were induced to consume appreciable quantities of the diets, the results were completely negative Despite the fact that the amino acid content of M-2 was not dissimilar to that of A-3 as shown in Table VII, in no case was cure initiated It was necessary to transfer the subjects to a skim-milk diet in order to arrest the downhill progression of the disease The explanation of this apparent paradox was found in the nitrogen balance data, a typical example of which is shown in Table VIII

As can be seen from these data, 85 per cent of ingested nitrogen is excreted in the feces *The obvious explanation is a failure* in digestion of the corn protein, and this despite the fact that skim milk and casein were most efficacious in the initiation of cure

TABLE VI

COMPOSITION OF FORMULATIONS

	Gm/Kilo of Formulation M 2	S 1	A 3
Amino acid mixture A 3			160
Corn protein concentrate	420 0		
African corn meal		835 0	
Glycine		25 0	
L lysine HCl	13 6	6 0	
DL alanine		25 0	
DL tryptophan	2 3	1 5	
L isoleucine c D alloisoleucine	10 4	5 5	
Salt mixture	80 0	80 0	96
Vitamins	2 0	2 0	2
Glucose	471 7	20 0	742
	1000 0	1000 0	1000

Acceptance of this premise forces a change in our concept of the etiology of the disease There can be no denying that an amino acid deficiency or imbalance may be the primary cause of the syndrome The efficacy of intact proteins of high biological value, however, seemed

TABLE VII

AMINO ACID COMPOSITION OF FORMULATIONS

	Gm/Kilo of Formulation A 3	M 2	S 1
Arginine	10 3	6 1	3 3
Histidine	5 2	3 4	1 9
Isoleucine	7 2	(7 2) 6 5*	(3 0) 3 3*
Leucine	16 2	17 6	(3 0) 8 0
Lysine	16 2	15 1	6 0
Methionine	3 5	3 5	1 2
Phenylalanine	13 7	10 2	4 2
Threonine	7 2	5 8	2 4
Tryptophan	2 5	1 8	1 2
Tyrosine	25 7	2 4	1 4
Valine	5 1	9 3	4 2

*The values in parentheses represent the isoleucine contents of the corn samples as determined by microbiological assay The figures in columns are those obtained by adding 15% of the microbial assay value to the supplemental L isoleucine

TABLE VIII

NITROGEN BALANCE — M 2 FORMULA

		Weight of Infant — 7 kg Nitrogen in mgms	
Intake	Fecal	Urinary	Balance
1261	1020	691	-450
1165	1068	763	666
1312	1023	850	552
1085	1127	750	-792
1088	908	654	-474
1073	942	784	653
1572	1190	612	230

to indicate that an actual dietary deficiency was the most important factor involved Now we must consider that this factor is of only secondary importance and that a controlling factor may be the unavailability of the amino acids or the low-quality protein of the patients' natural diet

Kliger and Kreh *(22)* reported some years ago that zein is not adequately digested in the growing rat large amounts of nitrogen appearing in the feces Later Geiger and his associates *(23)* showed that amino acid supplementation of enzymatically digested zein failed to support growth in rats although identically supplemented acid hydrolyzed zein was effective Both Drs Feirer and Seeley have observed that corn protein is rendered soluble with great difficulty by proteolytic enzymes

In the Merck laboratories we have recently found that although casein is readily hydrolyzed by commercial pancreatin zein is almost completely resistant to such action, possibly because it becomes gummy and coalesces into an intractable state If the zein is first treated with pepsin at pH 1 7, it remains granular and can be further attacked by pancreatin If, however the pepsin treatment is carried out without provision for maintenance of the proper high hydrogen ion concentration, almost no digestion occurs

These facts are of some interest in light of the following observations In a very limited number of cases we have found that administration of pepsin and pancreatin to kwashiorkor patients receiving corn protein did not prove beneficial Jayasekera *(24)* has reported that free acid and total acidity of kwashiorkor patients to be low whereas the peptic activity appears to be unaffected It is within the realm of possibility that increasing the gastric acidity of some of these subjects may enable them to utilize more efficiently the plant proteins of their diet Such a possibility may be tested experimentally

Granting for the time being a lack of high-quality animal protein in the diet and a failure of digestion of dietary plant protein to be the causes of kwashiorkor in its acute stage, we have yet to determine the

factors involved in precipitating the disease Most children in regions in which kwashiorkor is endemic pass through what appears to be a mild form of the disease Many of these children recover and lead reasonably normal lives without change of diet Why do others enter an acute stage from which there is no recovery without proper therapy? Is it because some infants are removed too abruptly from the breast or at too early an age? It is a well-known fact that children and the young of other species have low concentrations of gastrointestinal enzymes *(25 26 27)* Or does a severe infection at a critical time precipitate the disease? Certainly infections are widespread in these areas It has been established that some infections produce achlorhydria *(28)* These and other possibilities undoubtedly exist Only much continued experimentation will reveal the truth

In conclusion, we should like to consider possible developments in human use of amino acid mixtures You are all aware of Dr Allison's recent observations that addition of Dl-methionine and glycocyamine to the diets of tumor-bearing rats greatly increased the ratio of body-weight gain to tumor-weight gain *(29)* He has also shown that such supplementation increases the production of serum albumin Dr Chow *(30)* some years ago found that one protein hydrolysate would preferentially increase serum albumin in dogs, whereas a second caused increases in both albumin and globulin Dr Silber *(31)* observed that an amino acid mixture devoid of arginine was the most effective mixture tested in the regeneration of liver protein Dr Greenstein *(32)* has reported that arginine will detoxify ammonia All these observations may have practical applications in human therapy We believe the time is approaching when imbalanced amino acid mixtures will be widely used for treatment of specific disorders Certainly the phenylalanine-deficient preparation now being used in phenylketonuria is a highly imbalanced mixture Dr Armstrong has observed that a high percentage of the inmates of a Utah mental institution excrete abnormal indole metabolites Only time will tell whether tailor-made amino acid mixtures will benefit these people When the subject of amino acid imbalance is broached we should cease being alarmed, but be thankful that such imbalances do exist They undoubtedly can be dangerous, whether existing in nature, as Dr Allison has pointed out, or whether they are man made but their potential for good is great All we have to do is to find out how to use them

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6

AMINO ACID METABOLISM IN THE CENTRAL NERVOUS SYSTEM

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Until recently amino acid and protein metabolism in the central nervous system have usually been discussed in only a few paragraphs Our knowledge of this subject is still far from complete, but it is now possible to review many facts plus a number of reasonable inferences which begin to form a coherent picture

The central nervous system presents several special problems Its proteins are relatively more complex than those of many other organs, due to their close association with the neural lipids In organization the brain has a distinct heterogeneity Gray and white matter are further subdivided in cytological characteristics, in functional activities, and in chemical constitution To some extent these are problems which are met in all tissues, but the central nervous system is also distinguished by its isolation or protection behind the blood-brain barrier mechanism This imposes certain peculiarities in metabolism which have greatly complicated studies of cerebral amino acid and protein metabolism *in vivo (152)*

The fact that such metabolism can now be discussed in some detail is the result of much painstaking elucidation of the enzyme systems and metabolic sequences they subserve in relation to the functioning of the blood brain barrier The application of isotopic techniques, pioneered by Hevesy, Schoenheimer, and others, has provided insight into these problems, especially for the intact, functioning organism *in vivo* which would be difficult or impossible by other available approaches *(109)*

A review of all recent data on cerebral amino acid and protein metabolism would be beyond the scope of this paper However, consideration of a number of important aspects and findings will serve to emphasize the main principles and provide a basis for understanding new developments and derangements of normal metabolism

FREE AMINO ACIDS

Cerebral Concentrations

Amino acids account for about 40 per cent of the total solids of the brain *(82)* Most of them are protein-bound, but in contrast to most important cerebral nutrients which are present in only trace amounts,

free amino acids represent a substantial fraction of the solutes or ex tractives of neural tissues The total nitrogen of the brain is approxi mately 2 per cent of its wet weight *(58)* Of the total, 20 per cent is nonprotein nitrogen, half of which is composed of α-amino compounds, and much of the remainder includes immediate derivatives thereof *(6 151, 155)* The constitution of this free amino acid pool, which reflects to a considerable extent the relative importance of various amino acids in tissue metabolism, is summarized in Table I These data are taken primarily from analyses of cat tissues by Tallan, Moore, and Stein *(122)* examined under identical conditions Observations by many investigators on other species, including man, indicate that the values given in Table I are essentially representative of mammals in general

The amino acids in Table I have been divided into three groups on

TABLE I

FREE AMINO ACIDS IN TISSUES OF THE CAT

	(μM/g or ml)				
	BRAIN	LIVER	KIDNEY	MUSCLE	PLASMA
Group I					
Glutamic acid	8 7	2 9	7 1	2 45	0 1
Glutamine	5 3	3 7	2 4		
Aspartic acid	2 25	0 85	0 55	0 3	0 075
γ Aminobutyric acid	2 3	<0 1	0	0	0
Serine	0 7	0 35	0 4	0 5	0 2
[N Acetylaspartic acid]	5 9	0 1	0 1	0	0
[Ethanolamine (total free)]	6 45	1 7	2 1	0 2	0 01
Group II					
Taurine	1 9	8 3	3 55	4 45	0 055
Glycine	1 35	1 2	1 9	0 9	0 3
α Alanine	0 95	1 85	2 25	2 8	0 8
[Glutathione]	0 9	3 85	2 0	0 95	-
Group III					
Tryptophan†	0 035	0 05	0 035	0 075	0 01
Phenylalanine†	0 06	0 1	0 1	0 06	0 055
Tyrosine*	0 065	0 1	0 1	0 075	0 07
Methionine†	0 1	0 6	0 075	0 025	0 025
Cysteine*	?0 04				
Threonine†	0 2	0 25	0 3	0 35	0 1
Proline**	0 15	0 25	0 4	0 3	0 2
Ornithine *	0 045	0 15	0 045	0 03	0 015
Arginine	0 08	0 01	0 07	0 15	0 08
Histidine†	0 06	0 6	0 15	0 25	0 09
Lysine†	0 15	0 25	0 25	0 4	0 2
Valine†	0 2	0 35	0 55	0 2	0 2
Leucine†	0 15	0 25	0 25	0 15	0 15
Isoleucine†	0 1	0 15	0 15	0 15	0 05

†Amino acids considered essential or indispensable
*Amino acids which may be indispensable under certain conditions
**Hydroxyproline and citrulline are also present in brain

(References *71 10⌊ 111 121 123 128 132*)

the basis of their concentrations in brain Those in Group I are present in the highest concentrations and are more abundant in brain than in any other major body tissue Two derivatives of this group, N-acetylaspartic acid and ethanolamine, are included because of their relatively high cerebral concentrations Amino acids in Group II are also found in brain in appreciable amounts, but their concentrations in one or more other organs equal or exceed those for the central nervous system None of the so-called "essential or "indispensable" amino acids are found in either of these two groups They are all in Group III, which is composed of those free amino acids present in brain in much smaller amounts In general this latter grouping also applies to other tissues

Approximately 75 per cent of the total free amino acids of brain are contributed by members of the glutamic acid-aspartic acid group The prominence of this small number of amino acids in the free pool of the brain contrasts distinctly with other organs of the body This situation is not peculiar to the cat Similar data for glutamic acid and glutamine in other species are shown in Table II Less complete observations on

TABLE II

TISSUE CONCENTRATIONS OF FREE GLUTAMIC ACID AND GLUTAMINE FOR VARIOUS SPECIES

	(μM/g or ml)					
	BRAIN		LIVER		KIDNEY	
SPECIES	G COOH	G $CONH_2$	G COOH	G $CONH_2$	G COOH	G $CONH_2$
Man	10 0†	4 55†				
Cat	9 55†	5 3†	2 9	3 7	7 1	2 4
Sheep	10 5†	3 8†	5 8	1 7	6 3	1 0
Rat	10 4	4 0	3 3	3 8	5 3	1 5
Mouse	11 4	4 7	1 3	2 4	5 3	0 7
AVERAGE	10 35	4 5	3 3	2 9	6 3	1 4

†For cerebral cortex others not specified
G COOH glutamic acid G $CONH_2$ = glutamine
(References *71 111 127 129*)

free γ-aminobutyric acid *(98 132)* N-acetylaspartic acid *(121)* and aspartic acid *(132)* indicate that they also exhibit the same cerebral prominence in other species, including man

Another 15 per cent of the total free amino acids of brain are composed of the remaining members of Groups I and II in Table I Thus, all but nine of the amino acids comprise only 10 per cent of the total free cerebral pool and the "essential" or "indispensable" group represents less than half of this fraction It is characteristic of central

nervous tissues that their store of essential and important nutrients is small indeed Most body tissues have ready access to circulating supplies of amino acids Increases in free levels have been demonstrated experimentally after administration of large oral or parenteral doses of a number of individual amino acids In the case of the central nervous system, the interposition of the blood-brain barrier prevents ready access of all but a few amino acids from the cerebral circulation under the same experimental conditions

Access to the Central Nervous System

With the exception of glutamine *(66 111 126 132)* and asparagine *(132)* no significant increases in brain or cerebrospinal fluid levels can be demonstrated for most amino acids after large oral or parenteral doses This statement applies to glutamic acid, γ-aminobutyric acid, serine glycine, alanine, methionine, lysine, histidine, tyrosine, and tryptophan, and to protein hydrolysates *(39 66 83 99 111 126)* This does not mean that most amino acids fail to enter the central nervous system at all In phenylketonuria, where serum phenylalanine levels are markedly elevated, the cerebrospinal fluid level is also significantly increased *(23)* Exchange of glutamic acid from cerebral blood to the central nervous system compartment has been reported *(3)* And numerous studies with isotopically labeled amino acids indicate that probably all penetrate the barrier to some extent Results with labeled glycine tyrosine, tryptophan lysine, and methionine support this view *(40 47 53 73 107 124 160)* Thus, the adult central nervous system has a mechanism for acquiring amino acids from the cerebral circulation Because of the apparent inability to obtain significant increases in levels of the amino acids in brain, the mechanism of exchange seems to overshadow any actual net uptake mechanism

In the fetal and neonatal mammal the blood-brain barrier is absent or incompletely developed *(57 152)* At these stages net uptake of amino acids such as glutamic acid lysine, and methionine, can be demonstrated experimentally *(47 57 73)* The central nervous system undergoes rapid growth toward maturation during the late fetal and early neonatal period At this time there is little or no demonstrable operation of the blood-brain barrier, since electrolytes and amino acids enter the brain as readily as other body tissues Shortly after birth penetration of solutes into the central nervous system is markedly and progressively reduced The reduction in amino acid penetration parallels that of chloride, potassium, and thiocynate *(152)* As a result, the central nervous system at maturity is in a sense, isolated from the rest of the body except for a few substances such as oxygen, glucose, and glutamine Functionally the blood-brain barrier may be considered a homeostatic mechanism which stabilizes the neuronal milieu *(136 152)* The metabolic consequences of the barrier development will be discussed below

Metabolism in Cerebral Tissues

The principal aspects of the metabolism of many of the important amino acids in the central nervous system have been demonstrated experimentally That of others can be reasonably inferred from studies on other tissues Certain amino acids of particular significance to the central nervous system will be considered here in some detail

PHENYLALANINE and TYROSINE The presumed metabolism of these two amino acids in brain is summarized in Figure 1 Phenylalanine is certainly supplied to the brain as such, and tyrosine is probably similarly supplied, since its synthesis from phenylalanine has been

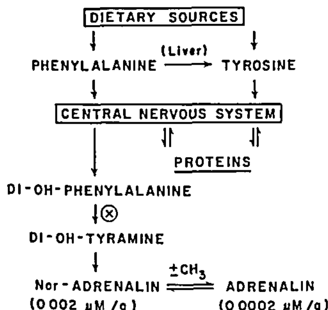


Figure 1 Phenylalanine metabolism in the central nervous system (see text for discussion) The step denoted by X requires pyridoxal phosphate as coenzyme

found to occur only in the liver *(141, 142)* The penetration of each into the central nervous system has been observed *(23 160)* The majority of the amounts supplied to the brain is incorporated into proteins

A small proportion of phenylalanine is almost certainly converted directly (or via tyrosine) into noradrenalin and adrenalin, as indicated in Figure 1 Both neurohumors are present in central nervous tissues, especially in the hypothalamus, in concentrations which are consistent with their production *in situ (21 147)* Direct demonstration of this metabolic sequence in cerebral tissues has not been reported, but it does occur in the adrenal medulla *(67 76 143)* Brodie and his associates have suggested that the central action of chlorpromazine is

mediated by its blockage of noradrenalin action and that the central effects of cocaine and similar agents are based upon activation of cerebral sympathetic systems (21 88)

Another aspect of phenylalanine illustrates several important factors in cerebral metabolism As a result of genetic absence of the enzyme catalyzing conversion of phenylalanine to tyrosine, the clinical syndrome of phenylketonuria occurs (64 140) Such patients exhibit mental retardation, seizures, elevated levels of phenylalanine in body fluids, and urinary excretion of large amounts of phenylpyruvic acid and related compounds (8 9 62 63) Some aspect of the abnormal metabolism of phenylalanine exerts a toxic effect on the central nervous system, probably during a critical postnatal period between 6 and 30 months of age (7) This biochemical lesion illustrates the importance of hepatic metabolism and function to cerebral metabolism and activity The dependence of the brain upon proper hepatic function has examples in phenylketonuria, galactosemia, hepatic coma, and hepatolenticular degeneration (Wilson s disease)

In the dietary treatment of phenylketonuria, evidence for the indispensable" nature of phenylalanine and, in its absence, of tyrosine for human nutrition has been obtained On a low-protein diet the temporary biochemical improvement in phenylketonurics is followed by reelevation of serum phenylalanine levels and resumption of phenylpyruvic acid excretion (91) Penrose and Quastel concluded that body proteins were being degraded to provide essential amounts of phenylalanine (91) Current diets, which are produced free of phenylalanine (and tyrosine), must be supplemented with minimal amounts of phenylalanine and with tyrosine to maintain optimal nutrition (9 17)

TRYPTOPHAN The three important functions of this "indispensable" amino acid are outlined in Figure 2 Part of the nicotinamide requirements of the body is derived from tryptophan (22 33 85) There is no evidence that this conversion can occur in neural tissues Nicotinamide is essential to cerebral metabolism as the functional component of the pyridine nucleotides (DPN and TPN), which form the first link in the hydrogen (or electron) transport system of intermediary metabolism The neural symptoms of pellagra are the consequence of failure of this coenzyme function of nicotinamide Tryptophan is also a component of cerebral proteins, and its penetration into the central nervous system has been demonstrated (107 139)

The system for the conversion of tryptophan to 5-hydroxytryptamine, or serotonin, is active in cerebral tissues (45, 139) Serotonin is present in various brain areas, particularly the midbrain and hypothalamus (5 20 90 138), and it has been proposed as a third neurohumor (44 92 163) Several groups of investigators have suggested that the mental disturbances produced by lysergic acid diethylamide (LSD) and related compounds result from interference with the action of serotonin (44 46 114 163) and that the tranquilizing action of reserpine may be mediated by its release of serotonin from its "bound" or stored form (90 92)

METHIONINE and CYSTEINE The metabolism of methionine in the

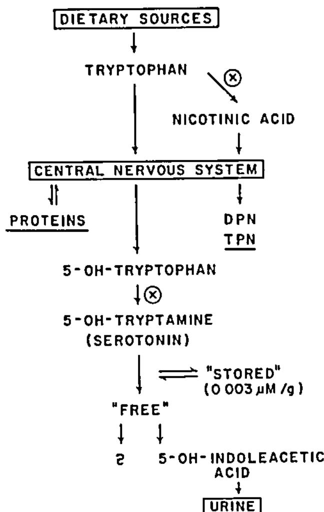


Figure 2 Tryptophan metabolism in the central nervous system The steps denoted by X require pyridoxal phosphate as coenzyme DPN and TPN = diphospho and triphos phopyridine nucleotides respectively

central nervous system is summarized in Figure 3 Penetration of this indispensable amino acid into brain has been clearly demonstrated *(40 47 124)* Methionine enters three important metabolic pathways It is incorporated into cerebral proteins Its methyl group is utilized via transmethylation to complete the synthesis of at least three important neural constituents, adrenalin (Figure 1), creatine *(19 25)* and choline *(144)* None of these transmethylation reactions have been directly demonstrated for brain, but indirect evidence is consistent with their occurrence therein

Methionine also undergoes conversion to cysteine, as shown in Figure 3 Only the sulfur atom of methionine is retained in cysteine, the remainder of the molecule being contributed by serine The carbon

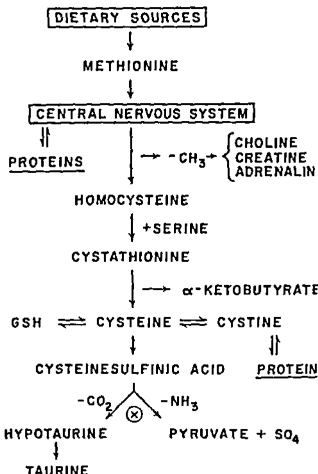


Figure 3 Methionine and cysteine metabolism in the central nervous system The steps denoted by X require pyridoxal phosphate as coenzyme GSH = glutathione

skeleton of methionine is removed as α-ketobutyric acid into fatty acid metabolism This series of reactions has been observed in brain for both the sulfur *(47)* and serine *(87 162)* moieties of cysteine

Some aspect of methionine metabolism is particularly important to the central nervous system, since small doses of its antimetabolite, methionine sulfoximine, produce seizures in all mammalian species *(52 86 95 135)* Massive doses of methionine will protect from or terminate such seizures *(95)* Methionine sulfoximine also inhibits glutamine synthesis in various tissues, including brain *(117 128 129)* and the seizures it induces can be terminated by administration of glutamine or asparagin to such animals *(131)*, although these compounds do not correct defective glutamine synthesis *in vitro (128)*, *(129)* The exact mechanisms involved are not yet understood

Cysteine also undergoes three metabolic reactions It is a component of the tripeptide, glutathione, which is an important coenzyme in

intermediary metabolism and a prominent cerebral amino acid compound (Table I) Two molecules of cysteine can condense to form the disulfide amino acid, cystine, which is important in protein structure Finally cysteine is degraded through cysteinesulfinic acid to either pyruvate plus inorganic sulfate by transamination or taurine by decarboxylation The enzymes and components of these reactions have been extensively studies in brain *(11, 15 28 29 34 101 132)* One of the end products, taurine, is a prominent amino acid compound in cerebral tissues (Table I) of all species, including man *(132)*

SERINE and GLYCINE With these two amino acids, the discussion turns to amino acids which do not have to be supplied in the diet The metabolic reactions of serine and glycine are outlined in Figure 4 Experimental evidence is clear that, under normal circumstances, glycine is derived mostly from serine and that serine in turn is derived from glucose *(79 105 116 162)* The pathway from glucose, via glycolysis, to glyceric acid (or phosphoglyceric acid), hydroxypyruvate, and serine has not been demonstrated in detail for cerebral tissues, but the overall conversion of glucose to serine has been established *(116 162)*

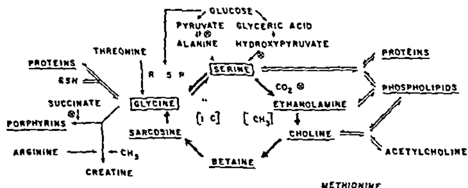


Figure 4 Serine and glycine metabolism in the central nervous system (see text for discussion) The one carbon cycle is indicated by the heavy arrows in the center of the figure The steps denoted by X require pyridoxal phosphate as coenzyme Ru 5 P = ribulose 5 phosphate GSH = glutathione

Glycine can also be derived from threonine *(156)* or from oxidative metabolism of glucose, via ribulose 5-phosphate *(157)* but neither of these alternatives compares in importance with serine Conversion of glucose to glycine in brain has been demonstrated *(116 162)* and the penetration of glycine into the central nervous system *(53)* as well as its conversion therein to serine *(103)* have been observed

Both serine and glycine participate in several important metabolic reactions in brain, in addition to their incorporation into cerebral proteins Serine and its derivatives, ethanolamine and choline are integral components of their respective phosphatides in lipid structures of the

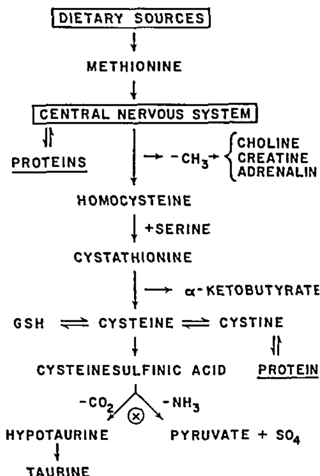


Figure 3 Methionine and cysteine metabolism in the central nervous system The steps denoted by X require pyridoxal phosphate as coenzyme GSH = glutathione

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alanine, glutamic acid, glutamine, γ-aminobutyric acid, and aspartic acid can all be derived from glucose both *in vivo* and *in vitro* *(14 26 27 87 116, 151 162)* As indicated in Figure 5, they are produced via the pyruvate, α-ketoglutarate, and oxalacetate stages of intermediary carbohydrate metabolism The individual reactions have all been shown to occur in brain *(30 32 69-71 98 150 151 153-155)*

A more detailed consideration of the metabolic reactions of this group is given in Figure 6 Glutamic acid and α-ketoglutarate are the

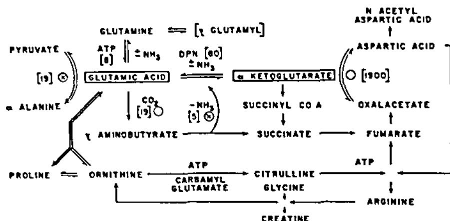


Figure 6 Metabolism of glutamic acid and aspartic acid in the central nervous system Coenzymes or cofactors for various steps are indicated as follows ATP = adenosine triphosphate DPN = diphosphopyridine nucleotide Co A = coenzyme A X = pyridoxal phosphate Curved arrows indicate transamination reactions The relative importance of various reactions may be in part inferred from the activities of the enzymes concerned shown in brackets in µM/g/hr of substrate removed or product formed *(81)* The metabolism of glutamine indicated to γ glutamyl denotes reactions concerned with either amide group or carbon skeleton transfers The incorporations of the amino acid compounds into proteins are not indicated in the figure but occur readily (see Figure 8)

key compounds in these reactions Glutamic acid can be formed from α-ketoglutarate either by direct amination or by transamination It is readily amidated to form glutamine, which enters into a number of important amide or γ-glutamyl transfer reactions Glutamic acid is a source of proline and ornithine and acts as a coenzyme in the conversion of the latter through citrulline to arginine, for which aspartic acid is also required Aspartic acid can enter an analogous set of reactions, including the formation of N-acetylaspartic acid, a compound apparently unique to neural tissues *(121 123)* The participation of glutamic and aspartic acids in the proline, ornithine, arginine cycles in brain can only be inferred from demonstration of isotopically labeled carbons in these compounds after glucose-C^{14} administration *(116 162)* and by analogy with other organs *(36 94 106 118)*

The metabolism of glutamic acid via γ-aminobutyrate to succinate is restricted to central nervous tissues *(10 16 98 100)* It occurs in

brain and spinal cord These reactions summarized in Figure 4, have been demonstrated in brain *(103)* In addition choline is essential for acetylcholine synthesis The acetylcholine system is present in all mammalian brains *(133)*, and interference with its proper functioning by alkylphosphate inactivators of acetylcholinesterase has serious peripheral and central neural consequences *(158 159)*

Glycine is an important structural unit for glutathione, for porphyrins, and for creatine, as shown in Figure 4 *(19 25 112 119)* None of these reactions have been established for brain tissues, but all three compounds are well represented therein, so that these reactions may well occur in the central nervous system

Glycine and serine are part of the so-called "one-carbon cycle," depicted in the center of Figure 4 Our knowledge about this cycle has recently been summarized by MacKenzie *(79)* It is concerned with the utilization of one-carbon formate, and methyl groups for which the folic-acid-vitamin-B_{12} group acts as coenzymes Several studies indicate that this cycle functions in cerebral tissues *(87 103 116 162)*

GLUTAMIC and ASPARTIC ACIDS The final amino acids to be considered belong to the glutamic-aspartic acid group, which, as indicated earlier (Tables I and II), comprises the majority of free amino acids in the central nervous system The metabolic interrelationships of this group are shown in Figure 5 There is good evidence that α-

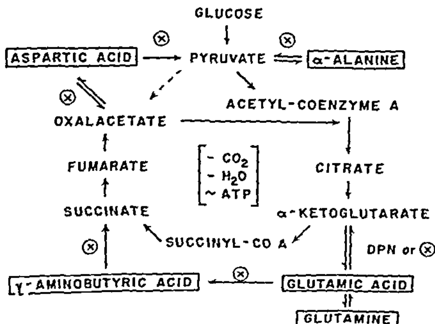


Figure 5 Metabolic interrelationships between the Krebs cycle of intermediary carbohydrate metabolism and the glutamic acid aspartic acid group of amino acids The end products of Krebs cycle operation are shown in brackets in the center of the figure Note the numerous steps requiring pyridoxal phosphate as coenzyme (denoted by X) DPN = diphosphopyridine nucleotide Co A - coenzyme A ATP = adenosine triphosphate

TABLE III

APPROXIMATE AMINO ACID COMPOSITION OF CEREBRAL PROTEINS

(μM/g Protein)

Alanine	159	Isoleucine	(109)
Aspartic acid*	224	Leucine	(203)
Cystine†	103	Lysine	(182)
Glutamic acid**	304	Methionine	179
Glycine	221	Phenylalanine	55
Proline	324	Threonine	216
Serine	213	Tryptophan	64
Arginine	155	Tyrosine	59
Histidine	198	Valine	(123)

Including asparagin
†Including cysteine
Including glutamine
Values in parentheses are approximate

(References *1 18 162*)

TABLE IV

PROTEIN FRACTIONS OF THE CENTRAL NERVOUS SYSTEM

A. Fractions by Isolation

1 Albumen
2 Globulins
3 Collagen and elastin (mainly nonneural elements)
4 Liponucleoproteins (account for majority of protein phosphorus)
  (1) Ribonucleoproteins (nucleolus and cytoplasm [Nissl])
  (2) Deoxyribonucleoproteins (nucleus)
5 Phosphoproteins
6 Proteolipids (major component of solids white gray ratio 4 1)
  (1) Type A (mixture 20% protein 80% lipid [*mainly cerebrosides*])
  (2) Type B (50% protein, 50% lipid [phosphatides and cerebrosides])
  (3) Type C (75% protein 25% lipid [phosphatides])
  (4) Neurokeratin (? proteolipid component)

B Fractions by Electrophoresis

		Gray (%)	White
1	Fast Component (prealbumen)	0 8	1 1
2	Albumen	1 9	3 0
3	α_1 Globulins (? lipoproteins)	4 2	7 2
4	α_2 Globulins (glycoproteins)	7 8	11 7
5	β Globulins (lipoproteins – ? proteolipids)	66 6	53 6
6	γ Globulins	18 9	23 4

(References *41–43 55 60 72 77 78 80 102*)

all mammalian species, including man *(56 98 132)* The intermediate compound, γ-aminobutyric acid, is found in appreciable concentrations in the brain (Table I) The significance of this uniquely neural sequence is not known, but the recent studies of Florey and associates *(13)* suggest that γ-aminobutyrate may function as an inhibitory or moderating substance for neural hyperactivity Pharmacological data which may be consistent with this view have been obtained *(56)*

PYRIDOXINE and AMINO ACID METABOLISM Before leaving the general subject of cerebral amino acid metabolism, the role of pyridoxine requires brief mention In Figures 1 to 6 the reactions catalyzed by pyridoxine in its coenzyme form of pyridoxal phosphate are denoted by the symbol X These reactions are numerous and important, as indicated by the consequences of pyridoxine deficiency The most outstanding neural accompaniment of pyridoxine deficiency in and mammalian species is seizures, but many species also exhibit peripheral neuropathies and demyelinating lesions in the spinal cord Pyridoxine is also unique among B-complex vitamins in that excessive amounts are toxic to the central nervous system, producing signs and pathological findings resembling those seen in its deficiency Since the coenzyme functions of pyridoxine are concerned principally, if not entirely, with amino acid metabolism, it is apparent that the proper functioning of these systems is essential to normal neuronal function and activity (For a review of pyridoxine in neural metabolism, see *130)*

PROTEIN METABOLISM IN BRAIN

So far the discussion has centered on the free amino acids of the central nervous system The majority of cerebral amino acids is bound in protein Recent studies have provided much new information about the metabolism of cerebral proteins, which is briefly summarized here

Protein Composition

The approximate amino acid composition of the total cerebral protein fraction is shown in Table III The tabulation indicates that virtually all amino acids are well represented in protein of the brain, but such data are not particularly informative because of the many protein fractions present and the heterogeneity of structure and function in various brain areas, which are presumably reflected in differences in protein composition

Proteins comprise about 8 per cent of the wet weight of the brain, with a distribution between gray and white matter of 45 per cent and 26 per cent respectively of the total solids in those divisions *(41 55 82 150 151)* Some idea of the complexity of cerebral proteins is indicated in Table IV, in which two different analytical approaches to this problem are presented No structure for any isolated cerebral protein has been determined, but an idea of the ability of neurons to synthesize such compounds is provided by the structure of the posterior pituitary hor-

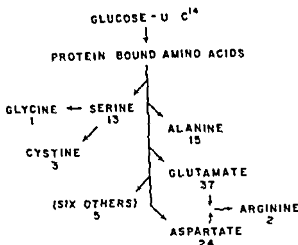


Figure 6 The conversion of glucose to protein-bound amino acids in mouse brain The figures are given in per cent of total carbon 14 activity in the cerebral protein fraction after intraperitoneal injections every 3 hours for 24 hours *(162)*

portions of "indispensable amino acids can be replaced by units derived from glucose under *in vitro* conditions Parallel studies on adult brain tissue showed labeling only in aspartic acid, glutamic acid, alanine, serine, glycine, and arginine *(116, 162)* It is important to note that these studies indicate an appreciable turnover or replacement of cerebral protein amino acids *in vivo* in a 24-hour period and that no net increase in concentration of any amino acid could be detected

Other investigations have demonstrated that protein turnover in brain is not limited to neonatal or immature brains The incorporation of L-methionine-S^{35} into various rat brain constituents *in vivo* has been studied by Gaitonde and Richter *(47)* Most of the experiments were carried out by intracisternal injection of the labeled methionine, since the relative specific activity in brain 15 minutes after injection by this route was almost twenty times that obtained after intraperitoneal injection It may be recalled that Friedberg and Greenberg *(39)* noted that, if the blood-brain barrier is by passed, proteins of brain incorporate more amino acids than liver, kidney or plasma proteins Most of the S^{35} activity in brain appears in the protein fraction distributed in a ratio of 3 1 between methionine and cystine At the same postinjection time liver had incorporated only 70 per cent as much In calculating the mean rate of methionine incorporation into protein, however, Gaitonde and Richter report that liver proteins incorporate it at four times the rate of brain proteins, when the half-life of the methionine bond in protein is about 14 days In studies on younger rats, these investigators observed a specific activity ratio for methionine in brain proteins twice that of mature rats studied under the same conditions

In unpublished experiments Lajtha, Furst, and Waelsch *(73)*[1] have

[1] The author is indebted to Dr Heinrich Waelsch for his kind permission to discuss these studies

mones, shown in Figure 7 The hormones are secreted by neurons of the supraoptic and paraventricular nuclei of the hypothalamus and carried down their axons to the posterior pituary lobe *(108)* On the basis of analyses of naturally isolated oxytocin and pitressin vasopressin

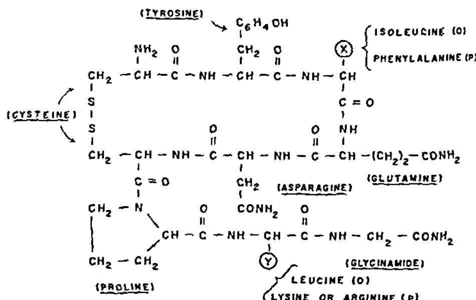


Figure 7 Structural formulae of the posterior pituitary hormones Distinctions between oxytocin (O) and pitressin vasopressin (P) depend upon the amino acids linked at points X and Y as indicated *(12 145 146)*

the structures have been determined and active, physiologically identical compounds have been synthesized in proof of these structures *(12 145 146)* Of course these hormones are not proteins but small peptide molecules The simplest proteins are some ten times as large, but the hormones demonstrate the synthetic capacity of neurons and exhibit some interesting structural features With the limitations in our knowledge of cerebral proteins, only the general characteristics of protein metabolism can be considered at present

Protein Turnover

The conversion of carbon-14-labeled glucose to amino acids of proteins in brain has been demonstrated both *in vivo* and *in vitro* *(116 162)* The results of such a study on the neonatal mouse *in vivo* are summarized in Figure 8 All the amino acids showing a significant degree of labeling are those known to be derived from carbohydrate metabolism *In vitro* studies on neonatal mouse brain resulted in the labeling of many more amino acids in the protein fraction leading the investigators to suggest that, at this period of brain development, certain

the blood brain barrier is progressively interposed, so that exchange now becomes predominant In this sense, the development of the barrier mechanism correlates with functional maturation of the brain With such maturation there is a shift in emphasis to the channeling of most metabolism through carbohydrate pathways It is probably more than coincidence that special enzyme systems, such as glutamotransferase and glutamine synthetase *(101)* and transaminases *(7)* appear or rise sharply in activity at this time The maintenance of continuing turnover or renewal of amino acid units of cerebral proteins becomes dependent on these systems concerned with amino group transfers in the mature brain It is in this area of cerebral metabolism that the glutamic-aspartic acid group plays a vital role

GLUTAMIC ACID AND CEREBRAL METABOLISM

The importance of the glutamic acid-aspartic acid group of amino acids in cerebral metabolism has already been indicated to some extent This group accounts for about 75 per cent of the total free amino acid content of brain (Table I) and is more predominant in brain than in other organs (Table II) It occupies a central position in the conversion of glucose to amino acids and in the transfer of amino groups (Figures 5 and 6)

Amino Acid Transfer

During the incorporation of labeled glucose carbons into cerebral proteins, the majority of labeling appears initially in this group of amino acids (Figure 8) This is also true for the free amino acid pool, as shown in Figure 9 These data are taken from studies on cerebral cortex *in vitro* but comparable results have been obtained *in vivo* *(26 98 162)* With either rat or human samples about one-third of the metabolized glucose C^{14} appears in the free amino acid fraction within 60 minutes *(14 120)* In the rat studies, approximately 50 per cent of the glucose was utilized and the distribution of activity in the free amino acid fraction was glutamic acid (plus glutamine) 56 per cent, aspartic acid 15 per cent, γ-aminobutyric acid 19 per cent, and α-alanine 10 per cent of the total activity in this fraction *(14)*

When pyruvate-2-C^{14} is incubated with rat cortex slices for only 20 minutes, 14 per cent is metabolized and 68 per cent of the total metabolized appears in the same free amino acids, as shown in Figure 9B *(27)* *In vivo* studies by the same investigator under comparable conditions show a similar distribution of the metabolized pyruvate within 3 minutes after intravenous injection (Figure 10) *(26)* When both *in vitro* and *in vivo* results for rat brain are compared with those for other organs, the importance of the glutamic-aspartic acid group in cerebral metabolism is readily apparent These data are summarized in Figure 11

Studies with rat cortex homogenates *in vitro* and rat brains *in vivo* using γ aminobutyrate as the labeled carbon source, yield essentially

obtained similar information on the metabolism of lysine by mouse brain The content of *free* lysine in the brains of immature mice is doubled after an injection of lysine into the whole animal, whereas only a small increase occurs in adult mice Using carbon-14-labeled lysine, a rapid exchange of lysine from blood to brain can be demonstrated The rate of exchange or flux is 0 7 to 5 2 μg/g brain/min and does not differ significantly for young and adult mice Thus, there is a replacement of half the *free* lysine in mouse brain within 45 minutes, which may be contrasted with a half-life for brain potassium of 24 hours In studies on the incorporation of lysine into cerebral proteins, Lajtha, Furst, and Waelsch *(73)* have observed a spectrum of turnover rates For the fraction with the fastest rate, the half-life is about two days, compared to about one day for liver proteins This rate, as indicated lysine, is not increased in the immature mouse In addition experiments on monkeys have revealed that protein turnover rates vary from brain area to brain area The proteins of the corpus callosum exhibit the shortest half-life, followed in order by cerebral and cerebellar cortices, subcortical areas, and spinal cord These investigators have also studied the cellular locus of lysine incorporation into proteins of monkey cerebral cortex The microsomal(Nissl) fraction exhibits a rate of lysine incorporation five times that of the total unfractionated cortex This finding is in agreement with results obtained on cells from other organs *(24 115)*

Despite the indications of active protein metabolism in brain, net synthesis of protein in adult brain may be a slow process In rats exposed to sublethal doses of GB (Sarin) gas, an alkylphosphate cholinesterase inactivator, the cholinesterase activity of brain was 2 7 per cent and of red blood cells 7 8 per cent of control values *(89)* and severe neurological signs including convulsions were observed Since it has been shown that such toxic agents permanently inactivate cholinesterase, so that it must be replaced by newly synthesized enzyme *(89 158)* the rate of regeneration of cholinesterase activity in these animals gives some indication of the ability of the brain to synthesize this enzyme protein In brain the rate of regeneration of cholinesterase activity was 3 7 per cent per day for the first 15 days and 0 4 per cent per day thereafter, compared to 4 4 per cent and 0 8 per cent respectively for the red cell cholinesterase activity The estimated times to regain control levels of activity were 140 (± 31) days for brain and 48 (± 6) for red cells in these rats *(89)*

It is obvious that the study of protein metabolism in brain presents many complexities but it is possible to draw several conclusions, particularly from the investigations of Waelsch and associates It is significant that the exchange of amino acids from blood to the brain pool appears to occur at about the same rate in neonatal and adult animals, although it is probable that renewal of the pools varies with each individual amino acid In neonatal brain however net uptake of amino acids appears to overshadow simple exchange in accord with the rapid growth of the brain during this period As the brain approaches maturation

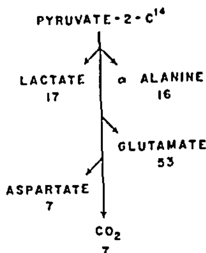


Figure 10 The utilization of pyruvate by rat brain *in vivo (26)* The values represent the per cent of total carbon 14 activity recovered in brain 3 minutes after intravenous injection Compare with *in vitro* studies (Fig 9B) and with results on other rat tissues (Fig 11)

active conversion of carbon chains in brain, amino transfers by the glutamic-aspartic acid group should be equally active and prominent Using N^{15}-labeled ammonium citrate or amino acids, such as glycine, a continuous interchange of amino and amide nitrogen in almost all amino acids of the body tissues has been found *(109)* The two compounds with the consistently highest N^{15} content are glutamic acid and aspartic acid *(109 110 113)* Under appropriate conditions high N^{15} content of amide groups can be demonstrated *(65 109)* indicating that

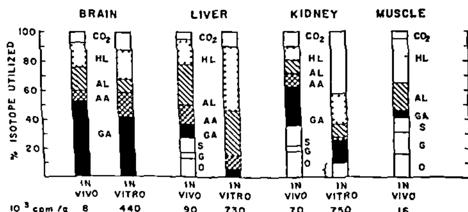


Figure 11 The distribution of carbon 14 labeling from pyruvate 2 C^{14} metabolized in various tissues of the rat 3 minutes after intravenous injection (*in vivo*) or 20 minutes after incubation of slices *(in vitro) (26 27)* Specific activities (cpm/g tissue) are given in each case at the bottom of the figure Abbreviations are as follows HL = lactic acid AL = α alanine AA = aspartic acid GA glutamic acid plus glutamine S = succinate G = glycogen etc O other tissue components (not identified)

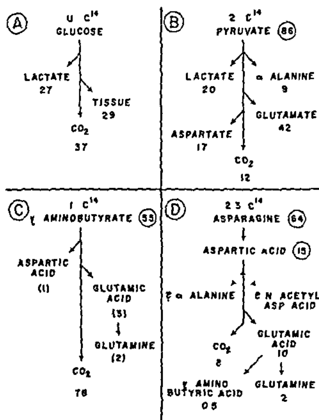


Figure 9 The metabolic importance of the glutamic aspartic acid group as interme diates in free amino acid interconversions demonstrated by *in vitro* isotope studies Ⓐ Utilization of glucose by slices of human cerebral cortex in 3 hours *(120)* Values are in per cent of labeled glucose metabolized See text for detailed data on rat cortex Ⓑ Utilization of pyruvate by slices of rat cerebral cortex in 20 minutes *(27)* The encircled number represents the percentage not metabolized Ⓒ Utilization of γ aminobutyrate by rat brain homogenates in 90 minutes *(98)* Encircled number rep resents the percentage not metabolized Of the amount metabolized 78 per cent was recovered as CO_2 and the rest by the amino acids in the order indicated by the numbers in parentheses (percentages not specified) Ⓓ Utilization of asparagin or aspartic acid by slices of cat cerebral cortex in 60 minutes *(132)* Encircled numbers represent percentage not metabolized Other values are per cent of labeled aspartic acid metab olized (CO_2 activity was not determined) Most of the asparagin metabolized was re covered as aspartic acid but labeling of other amino acids occurred in the same rela tive amounts as when aspartic acid alone was provided

the same results, as shown in Figure 9C *(98)* Similar data for cat cortex slices, incubated with asparagin or aspartic acid are given in Figure 9D These experiments indicate that the glutamic-aspartic acid group in brain is active and prominent in the metabolism of carbohydrate and amino acid carbon chains at all levels in the metabolic sequence

Comparable data for amino group transfers in brain are not available However, studies on other tissues indicate that, in view of the

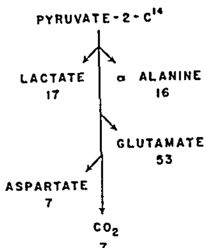


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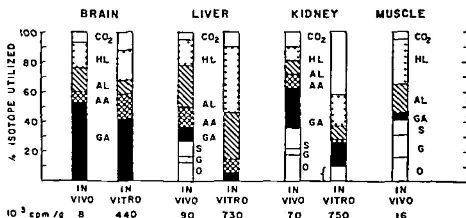


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glutamine is also actively concerned in nitrogen transfers Studies with glutamine-amide-N^{15} have shown that its amide nitrogen is handled like amino-N^{15} rather than ammonia-N^{15} *(59)* On the basis of the high activity of glutamic acid, aspartic acid, and glutamine enzymes in brain, it is likely that nitrogen transfers, both amino and amide, are very active in cerebral tissues Direct studies on these points should prove interesting and informative

Energy Metabolism

The importance of the glutamic-aspartic acid group encompasses more than its immediate role in amino acid and protein turnover It is also concerned with energy and possibly with activity metabolism of the central nervous system Since the original observation of Quastel and Wheatley *(93)* that, out of thirteen amino acids tested, only glutamic acid could be oxidized by brain tissue and support its respiration (oxygen uptake) *in vitro* glutamic acid has occupied a special position in cerebral metabolism Its ability to support brain respiration is due to its strategic location in relation to the Krebs cycle (Figure 5) and its ready conversion either by transamination or dehydrogenation to α-ketoglutarate (Figure 6) As a result there is a potentiality for energy yield in the form of high-energy phosphate bonds ($\sim$P) from the further metabolism of a α-ketoglutarate and succinate, so-formed, in the Krebs cycle *(75)* This potentiality together with the calculated theoretical energy yields are summarized in Figure 12

Compared to glucose oxidation, the energy yield is small Most investigators consider these reactions as emergency mechanisms which are poorly equipped to cope with demands of increased neuronal activity or to promote high-energy phosphate-bond production *(151 154 155)* This appears to be true with the exception of primate brains (however, see *75)* Here as shown in Table V, McIlwain *(81)* has found that, in addition to its ability to support the *in vitro* respiration of human cerebral cortex slices under resting conditions glutamic acid would support the increased respiration induced by electrical stimulation Similar studies on rat and guinea-pig cortex did not show this latter effect and results with monkey cortex were much smaller than those obtained with human samples *(81)* It is possible, therefore, that in primates, particularly man glutamic acid may subserve a secondary role in energy metabolism The observation that glutamic acid relieves hypoglycemic coma of patients or supports the activity of isolated perfused rat spinal cord may be consistent with this possibility *(84 137)*

Another facet of this same subject is the repeated observation that neuronal activity is associated with an increased production of free ammonia *(96 125 161)* This somewhat paradoxical fact may relate to recent studies by Vrba *(148 149)* and by Geiger and co-workers *(2 50 51)* When rats are forced to swim for 4 5 hours Vrba has reported that the amide nitrogen concentration of cerebral proteins is reduced the level of free glutamic acid is significantly lowered, and the level of

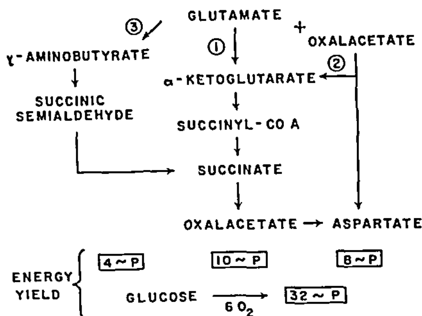


Figure 12 Theoretical yield of high-energy phosphate bond (~P) from metabolism of glutamic acid by glutamic (1) dehydrogenase (2) transaminase with oxalacetate or (3) decarboxylase The ~P yield from complete oxidation of glucose is given below for comparison See text for discussion

TABLE V

RESPIRATION OF CEREBRAL CORTEX IN VITRO
EFFECT OF SUBSTRATE ON RESTING AND STIMULATED METABOLISM

		(μM/g/hr)			
		HUMAN		GUINEA PIG*	
Substrate	Condition	Oxygen Uptake	Lactate Production	Oxygen Uptake	Lactate Production
Glucose 10 mM†	Resting	40 8	22 0	62 9	21 0
Glucose 10 mM§	Stimulated‡	78 4=	36 6	117 3	60 0
Glutamate 30 mM		52 5	-	40 9	

*Similar results for rat cortex Monkey cortex exhibited some degree of support by glutamate when stimulated

†Similar support (resting) by pyruvate (10 33 mM) lactate (30 mM) succinate (30 mM) fumarate (20 mM) glutamate (30 mM) and to some degree citrate (20 mM)

§Similar support (stimulated) by pyruvate and lactate but not citrate succinate or fumarate

‡Condenser pulses for 30 min at 18 v peak potential and time constant 0 4 msec

(Reference *81*)

free glutamine comparably elevated, and that these changes are reversed after a relatively short rest period *(148, 149)* On the basis of these findings, Vrba suggests that, during increased functional activity or excitation, cerebral proteins are deamidated and degraded, releasing amino groups which are incorporated by glutamic acid as glutamine During rest, glutamine is utilized for reamidation and resynthesis of protein This concept is consistent with the studies of Hyden *(61)* who observed changes in cytoplasmic nucleoproteins during neuronal activity

Geiger and co-workers have studied survival and activity of the perfused cat brain *in situ (50)* With a glucose free perfusate there is a decrease in cerebral nucleic acids (14 to 50 per cent), phospholipids (22 to 60 per cent), phosphoproteins (35 to 70 per cent), and nitrogen of the microsomal and soluble cell fractions (50 per cent) in 60 minutes *(2)* When glucose is supplied, none of these changes occur During the period of glucose-free perfusion, the excitability of the brain and its level of high-energy phosphate compounds are maintained, indicating that noncarbohydrate sources are energetically adequate *(2, 50, 51)* Geiger *(49)* has suggested that similar changes on a smaller scale may occur during neuronal activity (e g , in seizures) and that glutamic acid and glutamine are important intermediates in this type of tissue structure turnover

The significance and interpretation of these studies is difficult It is not clear whether the changes reported represent actual turnover of tissue components, notably proteins, during neuronal activity or simply reflect attempts to satisfy the increased energy demands in the face of inadequate carbohydrate supplies The latter interpretation is implied in Geiger's experiments and it could also apply to those of Vrba

Seizures

The importance of the several aspects of the glutamic-aspartic acid group in cerebral metabolism is emphasized by the situation in seizures Studies of the metabolism *in vitro* of cerebral-cortex slices from patients with focal cortical seizures have demonstrated significant derangements in acetylcholine, glutamic acid, and potassium metabolism *(127-129 134)* Similar defects have been found in cortical slices from animals in which seizures are experimentally induced *(128 129 135)* Restoration of such impaired metabolism to normal can be effected by the addition of glutamine, asparagine, γ-aminobutyric acid, α ketoglutarate, or adenosine triphosphate (ATP) to the incubation medium *(128 129 132, 135)* Glutamic acid is not effective in correcting defective acetylcholine metabolism *(135)* but has not been evaluated for other components of the biochemical lesion

Attention has been centered on the role of glutamic acid in seizures for several reasons In five seizure situations, glutamic acid levels in the brain are significantly decreased, as shown in Table VI Pyridoxine deficiency is associated with development of seizures in all species, including man *(130)*, and the coenzyme functions of pyridoxine in brain

TABLE VI

EFFECT OF SEIZURES ON CEREBRAL FREE GLUTAMIC ACID

Type of Seizures	Cause	Tissue Sample	Species	Glutamic Acid % of Control
Spontaneous	Epileptogenic focus	Cortex*	Man	51 5 (87)†
Induced	Methionine sulfoximine	Cortex*	Cat	54 5 (85)†
Induced	3 methyl 3-ethylglutarimide	Cortex*	Cat	10 5 (60)†
Induced	Fluoroacetate	Brain§	Rat	80 5
Induced	Strychnine	Brain§	Rat	70 0

*Incubated slices
†Frozen *in situ*
§Values in parentheses are % of control for initial samples prior to incubation

(References 35 54 56 127-129)

are closely linked with glutamic acid metabolism A role for γ-aminobutyric acid in inhibiting or moderating neuronal hyperactivity has recently been proposed *(13)* And the efficacies of glutamine, asparagin, γ-aminobutyrate, and its lactam, 2-pyrrolidinone, in correcting glutamic acid metabolism of epileptogenic cortex *in vitro* and controlling seizures *in vivo* in both experimental animals and man have been demonstrated *(56 128 132)* It should be recalled that derangement of methionine metabolism by methionine sulfoximine is associated with seizures and has been found to involve the glutamine-glutamic acid system *(128 129)* Abnormal metabolism of phenylalanine in phenylketonuria is also associated with seizures The mechanisms in this case are unknown, but interference of phenylpyruvic acid or phenylacetic acid with the Krebs cycle operation has been suggested *(68)* which could also interfere with glutamic acid metabolism secondarily

The data summarized above favor an intrinsic involvement of cerebral amino acids, particularly the *glutamic-aspartic acid group*, in seizure mechanisms Glutamic acid appears to have a central role, but recent studies suggest that aspartic acid and γ-aminobutyric acid, but not glutamine, are similarly directly involved *(56)* The significance of these findings is not yet clear It cannot be established from the present data that derangement of glutamic acid metabolism is *per se* a causative factor in seizures The biochemical abnormalities could result from the continuous hyperactivity Certain points seem to favor a closer association of the defects with initiation or perpetuation of seizure activity than with the results thereof These biochemical defects in epileptogenic cortex are chronic in contrast to the many transient changes which accompany overt seizure activity *(38)* yet they are readily reversible The rapidity with which pyridoxine administration alleviates seizures and electroencephalographic abnormalities in deficient infants *(31)* suggests correction of a causative biochemical

lesion In addition the efficacy of certain glutamic acid derivatives or analogs in protecting against induced seizures or controlling spontaneous seizures *(56 128-130)* may be of significance in this connection

If glutamic acid is concerned directly with the seizure process, it is still not clear what metabolic area is primarily concerned On the basis of the metabolic interrelationships of glutamic acid with oxidative metabolism in brain (Figure 12, Table V), an underlying defect in energy supplies or utilization might be favored This possibility has been discussed elsewhere *(129)* The studies of Vrba and of Geiger suggest an alternative mechanism concerned with protein and amino acid turnover during neuronal activity, although it has already been pointed out that this may be merely another facet of the energy-deficit concept The elucidation of these problems will provide information of importance not only to seizure mechanisms but to cerebral amino acid metabolism in general

CONCLUSION

Recent studies indicate that the turnovers of amino acids and of proteins in the central nervous system are active and of the same order as those of other organs of the body No evidence has been cited to suggest that, within the meanings used during this symposium, amino acid malnutrition or protein depletion affects the central nervous system No clinical signs of neurological dysfunction nor evidence of abnormalities in gross or microscopic examination of the brain have been observed in protein depleted dogs *(4)* or children dying of kwashiorkor *(48)* The mature mammalian central nervous system is isolated or protected behind the blood-brain barrier, which begins to function shortly after birth, more or less coincident with the time when myelination nears completion *(73)* The age at which barrier function becomes significant in man is not known, but the brain doubles in size between birth and about six months of age, during which time myelination is demonstrable in virtually all cerebral and cord areas *(74)* From analogies with other species, it is probable that blood-brain-barrier function in man assumes importance about this period It has been reported that starvation or malnutrition in rats does not interfere with the progress of myelination *(37)* It is possible therefore, that the central nervous system is preferentially spared under circumstances of amino acid malnutrition and that the barrier mechanism may play a role in this sparing If such is the case there may be implications for the nutrition of other organs, at least during the period of brain growth in the face of inadequate dietary protein intake A comparable preferential sparing of the central nervous system early in pyridoxine deficiency has been suggested *(130)*

The interposition of the blood-brain barrier between the cerebral circulation and the central nervous system has resulted in a specialization of metabolism The utilization of carbohydrate (glucose) predominates with the result that the glutamic acid-aspartic acid group of amino

acids becomes preeminent in the conversion of glucose to amino acids and protein and in transfers of amino nitrogen In addition, this group of amino acids appears to be important for the functional activity of neurons Glutamic acid is unique in its ability, particularly in primate brains, to support oxidative metabolism The presence of N-acetyl-aspartic acid and γ-aminobutyric acid exclusively in neural tissues suggests special functions in the central nervous system for the glutamic-aspartic acid group Finally, the intimate association of pyridoxine deficiency and of deranged glutamic acid metabolism with seizures implicates glutamic acid and related compounds in neuronal activity and excitability The proper maintenance of amino acid and protein metabolism in the central nervous system appears to be essential to the normal economy of the neuron and its metabolic and functional activities

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